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A CLINICO-PATHOLOGICAL AND EXPERIMENTAL STUDY OF THE FUNCTIONAL-STRUCTURAL RELATIONSHIP OF GOITER

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We propose to present data from both the experimental laboratory and the clinic, together with an analysis of the accumulated literature, in an attempt to further clarify the problem concerning the relationship between the function and structure of the thyroid gland. Extensive clinical and experimental investigations have been conducted with the view of proving or disproving the existence of such a relationship. It is of interest to scrutinize the evidence that has accumulated which may offer an explanation for these widely accepted and possibly correct clinical observations.

According to Warthin (1) there do not seem to be any histological criteria, so far as the thyroid epithelium is concerned, that give a pathologic unity to all the clinical forms of thyroid hyperplasia and dysfunction. Rienhoff (2) believes that "associated with a clinical remission, whether artificial or spontaneous, in cases of exophthalmic goiter, involutional changes in the histologic structure of the thyroid gland occur which are just as definite and fundamental as the microscopic alterations occurring during hypertrophy and hyperplasia. Areas of hyperinvolution correspond clinically and histologically to the so-called colloid adenomas, cystic adenomas, fetal and colloid or mixed adenomas and colloid cysts. The areas of hypoinvolution are localized encapsulated areas of persistent hypertrophy and hyperplasia. Furthermore, involution of the thyroid gland occurs following hypertrophy and hyperplasia whether this is physiologic or pathologic." Marine and Lenhart (3) are impressed with the frequency in which active hyperplasia of both the thyroid gland and lymphoid tissue are found in exophthalmic goiter. They believe, however, that these changes are not specific for this syndrome and that they may be seen in a variety of other abnormal body states. Reinhoff and Lewis (4) find that in all cases of clinical hyperthyroidism there is always hypertrophy and hyperplasia of the thyroid parenchyma regardless of whether

the gland is clinically nodular or the process diffuse. They also state that "in view of the fact that the hypertrophy and hyperplasia of the gland which invariably accompany clinical hyperthyroidism cannot always be distinguished histologically from the hypertrophy and hyperplasia of a compensatory type, attempts to draw clinical deductions from microscopic sections may lead to incorrect conclusions in many instances." According to Hertzler (5) the toxic stage of goiter is always associated with increased proliferation of the thyroid gland. He further believes that exophthalmic goiter differs from toxic adenoma only in degree and rapidity of development, but not in kind. Finally, Plummer (6) is of the opinion that exophthalmic goiter is a definite clinical complex always associated with hyperplasia of the thyroid, but should be differentiated from the constitutional states that may develop with non-hyperplastic goiter.

From the foregoing brief review of the literature, it seems that the weight of evidence does not point to the existence of any direct relationship between the function and structure of the thyroid gland, although most observers are in accord with the view that hypertrophy and hyperplasia of the gland are constantly present in cases of exophthalmic goiter.

From our clinical material we collected 544 consecutive cases of different types of goiter, which, for convenience of description, we have divided into three main groups, namely, (1) exophthalmic goiter, (2) nodular toxic goiter and (3) nodular and colloid non-toxic goiter. These subdivisions were based mainly upon the clinical picture presented by the patient, the rise in basal metabolism and the appearance of the gland in the neck.

All the thyroid specimens were obtained fresh in the operating room. The weight of the specimen was determined and gross peculiarities detected with the naked eye examination were recorded. A number of sections were then taken from various parts of each specimen, fixed in 4 per cent solution of formaldehyde, and then cut and stained with hematoxylin-eosin and Van Gieson stains. The latter staining method was also adopted in order to demonstrate more specifically the presence and extent of fibrous tissue formation. In studying the microscopic sections, particular attention was paid to the size, shape and distribution of the acini, the height and staining peculiarities of the epithelium, the character of the colloid and stroma, and the degree of lymphoid changes whenever present.

An analysis of the 544 cases at our disposal showed that 294 were of the exophthalmic goiter variety; 50 belonged to the toxic nodular goiter and 200 were classified as nodular and colloid non-toxic goiter.

Statistical Results. Of the 294 exophthalmic goiters, 222, or 75.5 per cent, showed definite evidence of anatomical changes in the gland indicative of heightened glandular activity. Clinically, these patients showed all the ear marks of thyrotoxicosis associated with bilateral exophthalmos; and anatomically, the glands were markedly hyperplastic. The thyroids were firm and beef-like in consistency, the cut surface was opaque and lost its

general translucency. Histologically, there was marked increase in the size of the epithelium, crowding together of the acini, softening of the colloid and increased vascularity of the stroma. The acinar epithelium showed marked proliferation as judged by the presence of large numbers of mitotic figures, piling up of the epithelium and the formation of spur-like projections into the lumen of the acinus. Lymphoid hyperplasia was also a conspicuous and frequent finding, showing in many instances well-formed lymphoid follicles with large germinal centers. One of the patients belonging to this group died shortly after operation because of a thyroid crisis, and at autopsy there was found a very large thymus which weighed 60 grams, in addition to numerous miliary abscesses in the liver. In the remaining 72 cases there was relatively little gross or microscopic evidence of hypertrophy or hyperplasia of the thyroid, although clinically these patients presented signs of thyrotoxicosis with exophthalmos.

The nodular toxic goiters numbered 50, and 42 or 84 per cent of these cases showed a marked relationship between the clinical and anatomical picture. Clinically, these patients presented evidence of a moderate degree of thyrotoxicosis with no ocular changes. Anatomically, the glands appeared to consist of multiple nodules closely crowded together and separated from each other by a scant amount of fibrous tissue. The parenchyma of the individual nodules showed definite hyperplastic changes, although occasionally there could be seen some involutional changes.

The nodular and colloid non-toxic group numbered 200, and 184 or 92 per cent of these patients presented a definite relationship between the anatomical and clinical picture. These patients showed very few toxic symptoms, and the basal metabolism was relatively low or even subnormal. The glands showed all degrees of degenerative changes such as fibrosis, necrosis with cyst formation, hemorrhages, calcification, and at times also, ossification. Active hyperplasia of the acinar epithelium was rarely encountered; and whenever present, it was scant and occurred in only small localized areas. The acini in the colloid form of goiter were very large and distended with either solid or edematous colloid. The epithelium was very low and compressed. In the nodular type, the acini were irregular in size and shape, the colloid edematous, and the epithelium low or flattened, undergoing in many instances extensive degenerative changes. The stroma consisted of diffuse and thick fibrous tissue formation, densely infiltrated with round cells. Lymphoid structures were not commonly observed here. It is of interest to note in this connection that portions of the normal thyroid tissue immediately adjoining a nodule appeared compressed and such patients frequently showed a very low or even subnormal basal metabolism, probably because of interference with the normal function of the compressed thyroid tissue.

From the results of these clinical and anatomical observations, we believe that there exists a certain parallelism between the function and structure of the thyroid gland. This relationship is perhaps less apparent in exophthalmic goiter but is very pronounced in the other forms of goiter.

EXPERIMENTAL STUDIES

In order to demonstrate still further the existence of a possible relatioinship between the function and structure of the thyroid gland, we resorted to the following experiments on guinea pigs. The thyroid of a normal guinea pig presents an anatomical picture which is quite similar to that of man. Histologically, such a gland may be visualized as consisting of medium-sized acini lined by low cuboidal epithelium. The acini are uniformly distributed and show little variation in size and shape. The stroma consists of loose wisps of fibrous connective tissue, and contains in it the blood vessels and lymphatics, and occasionally also, a few lymphocytes. The colloid is mostly solid and fills the entire acinar space. In the normal thyroid only a small number of the acinar epithelia show evidence of mitotic cell division, the number of mitoses in the entire gland not exceeding 200. The glands usually show a much lower figure, averaging a total of about 100 mitoses.

When potassium iodide is administered in graded doses to normal guinea pigs there results a marked proliferation of the acinar epithelium, leading to a definite increase in the number of mitotic figures (7). Thus, while the average number of mitoses in the total normal gland is about 100, the iodine-treated animal shows an increase to several thousands mitoses. In addition to the increase in the number of mitoses, the thyroid shows other histological changes characteristic of increased gland activity, namely, increase in the height of the epithelium, softening of the colloid and irregularities in the size and shape of the acini. Such animals also show evidence of a moderate increase in the functional activity of the thyroid as judged from the moderate increase in basal metabolism. The results of such experiments are suggestive of some relationship between the function and structure of the thyroid gland.

Marine, Rosen and Cipra (8) and Marine and Rosen (9) have shown that when immature rabbits are fed on alfalfa, hay and oats, and are simultaneously subjected to daily intramuscular injections of 0.05 to 0.1 cc. of methyl cyanide, there occurs in these animals a bilateral exophthalmos in association with thyroid hyperplasia. Loeb, Bassett and Friedman (10), Loeb and Friedman (11) and Rabinovitch (12) obtained very similar results in normal guinea pigs injected with a fresh preparation of the acid extract of the anterior lobe of the bovine pituitary. One of us (J. R.) has shown that the administration of such pituitary extract to normal guinea pigs causes characteristic changes in the thyroid which in many instances are analogous to those seen in the thyroid of Graves' disease in man. Within 3 or 4 days after the beginning of the pituitary injection, the thyroid of these animals becomes transformed into a histologically very active gland, showing a marked heightening of the acinar epithelium and a pronounced increase in the number of mitoses; the number exceeds in many cases the count of 50,000 as compared with the normal count of 100. Associated with this increased epithelial proliferation there

occur other structural changes characteristic of increased glandular activity. The acini become irregular in size and shape, and the colloid softens considerably. The epithelium tends to pile up and form papilla-like projections within the lumen of the acini. Associated with these proliferative changes in the thyroid there are also noted signs of increased functional activity of the gland. The animal develops a bilateral exophthalmos, becomes irritable, the pulse is accelerated and the basal metabolism takes a sharp rise; it may exceed several times the normal rate. It has also been shown that the distribution of the organic and inorganic iodine in the thyroid and in the blood of the pituitary-treated guinea pig becomes similar in ratio to that which occurs in Graves' disease in man (13). If potassium iodide is now given to these animals, the thyroid reassumes its normal histological appearance; also, the basal metabolism diminishes and the pulse rate decreases. Schockaert (14) reports similar findings in the thyroid of ducks which have been treated with extracts of the anterior lobe of the pituitary. This investigator noted a pronounced increase in the size of the thyroid amounting in certain instances to 60 times the normal. Histologically also, these thyroids showed marked glandular hyperplasia and presented a picture almost identical with that seen in human exophthalmic goiter. Such experimental findings support our contention that there exists a direct relationship between the structure and function of the thyroid gland.

SUMMARY

The results of a study of 540 cases of goiter are reported. In a large proportion of the 294 cases of exophthalmic goiter there was a definite relationship between the function and structure of the thyroid gland. In the more typical cases of exophthalmic goiter the changes in the thyroid were constant and quite characteristic. There was always evidence of marked glandular hyperplasia and increased lymph-follicle formation. In the other forms of goiter, diffuse or nodular, toxic or non-toxic, the relationship between the function and structure of the thyroid was also quite striking. A rather definite parallelism seemed to exist between the anatomical changes in the thyroid and the clinical picture manifested by the patient. Our clinical results are substantiated by experimental findings previously reported in animals. The administration of potassium iodide or acid extract of the anterior lobe of the pituitary to normal guinea pigs resulted in both an increased functional and hyperplastic change in the thyroids.

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SOME FACTORS INFLUENCING THE LENGTHS OF SURVIVAL FOLLOWING BILATERAL SUPRARENALECTOMY

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For any random group of animals, the period of survival following complete adrenalectomy varies over a rather large range. In 1926, Jaffe (1) reported that animals suffering from "acute or sub-acute insufficiency" died within 30 days after operation. His rats ranged in age from 30-59 days. Pencharz, Olmsted, and Giragossintz (2) reported survival periods ranging up to 18 days with an average of 7 or 8 days. Gaunt (3) used animals from five colonies. Four of these gave practically identical results with 95 per cent of the bilaterally suprarenalectomized subjects dying within 20.5 days, and with an average life span of 7 days. The fifth colony differed markedly. Only 50 per cent died within the upper limit of 30 days and the average survival period for these rats was 14.4 days. Gaunt also noticed that animals under two months of age had a life span of 5.7 days on the average (for the first four colonies), or 1.3 days shorter than the average for all the animals. Ingle (4) reported survival periods ranging from 4 to 30 days with a mean of 15 days for 32 adult rats. Kroc and Martin (5) working with a group of 35 female albino rats from 4 to 6 months old report survival periods ranging from 6-29 days with an average of 14.9 days. These figures are for two-stage operations. For those cases in which the operation was performed in one stage, the average was slightly lower. Firor and Grollman (6) discuss some of the factors influencing the length of post-operative survival, and mention pre-operative treatment, the anaesthetic and its duration, the age of the animal, the completeness of the extirpation and the post-operative treatment.

The present authors have attempted to discover some of these factors making for individual differences in survival period. One-stage operations were successful in 68 animals, i.e., these operations were followed by the usual symptoms of insufficiency, weight loss and death. Autopsy in 15 of these cases (22 per cent) selected at random proved that all adrenal tissue had been removed. In all surviving cases, however, hypertrophied adrenal cortex tissue was found at autopsy. The animals were divided into age groups as follows, one animal was taken from each litter at ten-day intervals beginning at the date of weaning, twenty days after birth. Data are available, therefore, for 6 age groups; those operated on at 20, 30, 40, 50, 60, or 70 days of age. Utmost precautions were employed to perform carefully aseptic operations under light ether anaesthetic. Pre-operative and post-operative living conditions for all rats were optimal as regards temperature, diet, living quarters, et cetera. Daily weight records were taken just prior to the evening feeding time.

RESULTS

A. *Effect of Age on Survival Period.* Firor and Grollman (6) state that "the age of the rat is a predominant factor in determining survival period." This conclusion was arrived at by using body weight as an index of age. The following table adapted from their report, summarizes their results in this connection. The authors conclude, "with increasing size (i.e., age) the survival period increases." A survey of the table reveals that all survival periods are rather short, and that a large number of rats did not survive even one day. In fact, all of their lightest group (i.e., youngest) succumbed in one day. It seems rather arbitrary to attribute

TABLE I

SHOWING THE EFFECTS OF WEIGHT (I.E., AGE) ON SURVIVAL PERIOD
(Adapted from Firor and Grollman)

Weight of Rat	Number	Average Survival Period	Range
Less than 50 gms.	6	1.0 days	0
50–75 gms.	14	2.0 days	1– 5 days
76–100 gms.	38	4.3 days	1– 9 days
101–150 gms.	48	7.0 days	1–18 days
151–200 gms.	20	8.6 days	1–20 days
Over 200 gms.	32	9.0 days	2–21 days

these deaths to adrenal insufficiency since the animals would scarcely have had time to recuperate from the surgical shock. We have treated our own data in the same way so that they may be compared to those of Firor and Grollman. Table II summarizes these data.

TABLE II

SHOWING THE EFFECTS OF WEIGHT (I.E., AGE) ON SURVIVAL PERIOD

Weight of Rat	Number	Average Survival Period	Range
Less than 50 gms.	16	5.7 days	4– 9 days
50– 75 gms.	12	6.3 days	3– 8 days
76–100 gms.	8	7.6 days	6–13 days
101–150 gms.	22	14.7 days	6–36 days
151–200 gms.	8	16.0 days	10–22 days

Although the same general conclusion may be inferred from this table, our survival periods are longer on the average than those of Firor and Grollman, and are more in line with the results of Ingle (4) and Kroc and Martin (5).

A more direct attack on the effects of age on survival period can be made by computing averages for each one of our age groups. The data thus derived are summarized in Table III. This table indicates the number

TABLE III

SHOWING EFFECTS OF AGE ON SURVIVAL PERIOD

Age in days	20	30	40	50	60	70
Number of cases	11	14	15	13	8	7
Mean survival period in days	5.6	6.2	8.3	14.9	14.4	17.0
Median survival period in days	5.5	7.3	8.1	13.0	14.5	18.8
Range in days	4–9	3–8	3–16	6–36	9–20	6–28

of animals in each age group, the average and median survival periods, and finally the range, for each of the six age groups. Inspection of this table reveals that the period of survival increases slowly from 20 through 40 days, rises rapidly during the next ten days and again increases slowly, with minor variations, in the last groups. Whether the period of survival will continue to lengthen after 70 days is conjectural. However, we would venture to suggest that it will vary around a mean of 15-20 days with marked individual differences.

The data presented here, and those presented by Firor and Grollman, reveal an unusual increase in the survival period from the 40-day group to the 50-day group. Since puberty occurs in the rat during this period, some relation between increased survival value and maturing sexual function is indicated.

B. *Sex Differences.* In order to find the effects of sex differences on length of survival period, it will be necessary to treat the data for each age group separately. Table IV compares the data on male and female rats, divided according to age groups.

TABLE IV

Age Group	Male Rats		Female Rats	
	Number	Average Survival Period	Number	Average Survival Period
20 days	6	5.00 days	5	6.40 days
30 days	6	6.17 days	8	6.25 days
40 days	7	8.28 days	8	8.37 days
50 days	5	13.20 days	8	16.00 days
60 days	6	15.00 days	2	12.50 days
70 days	4	15.25 days	3	19.33 days

In every age group but that comprising 60-day-old animals, females survive longer than males; and in that group the average for the females is based on only two rats, one of which survived only 7 days, probably because of rather severe hemorrhage at the time of operation. Although the indicated differences would not satisfy the rather strict criteria of statistical reliability, still the trend is consistent enough to merit attention. We feel safe in concluding that, other things being equal, female rats withstand slightly longer the effects of severe adrenal insufficiency.

C. *Effects of Initial Body Weight.* Since bilaterally adrenalectomized rats suffer marked weight loss during the survival period, the assumption that those rats which weighed the most at the time of the operation would survive the longest is tenable. Available bodily reserves, or general health and vigor might be presented as possible causal factors in the event such a finding were validated. It is also possible that the important age difference discussed above would depend entirely on the factor of initial weight, since there is, of course, a high correlation between age and weight. To solve this problem by experimental means would be difficult, since it would

be necessary to hold body weight constant and vary age. And any of the methods of controlling weight, such as diet restriction or thyroidectomy, would add a third complicating factor.

We have attempted to analyze our results in a way aimed at this question. And while the result is wholly unsatisfactory from the viewpoint of reliability, due to there not being enough animals to separate the sexes, we present them here as a hint for further research. The data in this case also are treated separately for each age group. Table V shows the average initial weight for each age group in the second column. The third and fourth columns give the average survival periods for those above and

TABLE V

Age Group	Average Initial Weight	Above Average % ♀	Above Average Survival Period	Below Average % ♀	Below Average Survival Period
20 days	30.40 gms.	66	6.33 days	20	4.80 days
30 days	55.20 gms.	33	6.66 days	75	5.87 days
40 days	93.06 gms.	43	10.42 days	62	6.50 days
50 days	123.90 gms.	0	13.20 days	100	16.00 days
60 days	162.60 gms.	0	15.60 days	66	12.33 days
70 days	170.60 gms.	0	14.25 days	100	19.33 days

below this average respectively. According to this table, the below-average rats in the first age groups succumb earlier than the above-average rats, while for the later age groups this relationship is just reversed. These differences cannot be accepted as due to body weight alone. Perhaps the important factor here again is sex. The figures in the third and fifth columns are the percentages of each of these categories that is composed of female rats. Thus the advantages are seen to follow female predominance more consistently than mere weight. Although the results are far from unequivocal, we would conclude that with age and sex constant initial weight has relatively little effect on the length of the survival period.

D. *Weight Loss.* From the daily weight records obtained, we have computed the average weight loss and the percentage weight loss for each of the six age groups. These data are given in Table VI.

TABLE VI

SHOWING AVERAGE WEIGHT LOSS AND PERCENTAGE WEIGHT LOSS FOR EACH AGE GROUP OF RATS

Age Group	Number of Animals	Average Weight Loss	Percentage Weight Loss
20 days	11	2.59 gms.	8%
30 days	14	1.25 gms.	2%
40 days	15	10.36 gms.	11%
50 days	13	5.42 gms.	4%
60 days	8	20.37 gms.	12%
70 days	7	27.36 gms.	14%

There does not appear to be any consistent relation between age and percentage weight loss for these animals except that roughly considered the older rats lose more than the younger rats, even relative to their initial weight. The relation between weight loss and length of survival period is

shown by the data in Table VII. This table gives the average length of survival for the rats losing more than the average, and for those losing less. The data for each age group are treated separately.

TABLE VII

SHOWING AVERAGE SURVIVAL PERIODS FOR THOSE RATS LOSING MORE THAN AVERAGE WEIGHT AND FOR THOSE LOSING LESS THAN AVERAGE

Age Group	More Than Average	Less Than Average
20 days	5.00 days	6.40 days
30 days	5.37 days	7.33 days
40 days	7.14 days	9.37 days
50 days	10.12 days	22.60 days
60 days	12.80 days	17.00 days
70 days	15.25 days	19.33 days

In every age group those rats that lose more than the average for that group in body weight also succumb sooner to the effects of severe adrenal insufficiency. This conclusion is, of course, not unexpected. Yet care must be exercised in its interpretation. We do not look for any causal relation between these two variables, but we suspect that they are merely concomitants, both depending on a third factor, which may be general vigor, health, or the severity of the operational shock.

CONCLUSIONS

Diverse factors appear to influence the duration of the survival period subsequent to complete adrenalectomy. (1) The period of survival varies directly with age. (2) Other things being equal, female rats tend to survive longer than males. (3) Contrary to a plausible assumption, survival period is not dependent upon initial body weight. (4) Rapid weight loss is symptomatic of short survival period. However, it is probable that both are incident to other factors not yet isolated. The most significant differences in survival period are those conditioned by age differences.

SUMMARY

One-stage adrenalectomies were performed on 68 rats. The animals were divided into six groups according to age at the time of operation—beginning at 20 days and proceeding at 10-day intervals through 70 days. Daily weight records were kept for each rat beginning with the day of operation. The data upon analysis showed the following: (a) Age is a predominant factor in determining the length of survival period. There is a gradual lengthening of this period for our three youngest groups (20-40 days) a quick jump to the 50-day group and a further gradual increase in the last groups. The possibility of a relation between increased survival value and onset of puberty is indicated. (b) As regards sex, there is a consistent advantage on the side of the females, although the differences are small. (c) Initial body weight, if isolated from age and sex differences, appears to have no influence on the length of post-operative survival. (d) Weight loss during the period of survival correlates directly

with length of surival period. Accordingly, rate of weight loss is a useful indicator of the severity of insufficiency. There may be still other factors influencing lengths of survival period, but the most significant factor indicated by this study appears to be the age of the animal at the time of operation.

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COWS' MILK AS A POSSIBLE EXCRETORY SOURCE OF THE ANTERIOR PITUITARY-LIKE HORMONE

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It has been shown that in human female pregnancies both the anterior pituitary-like hormone (AP-L) and the female ovarian hormone, oestrin, occur in abundant amounts both in the patient's urine and blood (1) (2) (3) (4). Even in non-pregnant women the presence of both hormones can be demonstrated in smaller amounts in the blood, and the ovarian follicular hormone in the urine (5).

In pregnant mares both the AP-L hormone and the ovarian follicular hormone are found in the blood and urine, whereas in pregnant cows the follicular hormone is found in the urine but the AP-L hormone is absent from both blood and urine (6) (7). This absence has never been explained even though it would seem that the presence of both hormones is as essential in pregnancy and in menstrual control in cattle as in humans, since the processes are almost identical.

The close association and relationship of the AP-L hormone and the follicular hormone in the human female as necessary factors in pregnancy and menstruation is generally admitted. That human milk contains the follicular hormone has been asserted (8) and has led to a consideration of the possibility that it might contain AP-L hormone also. Moreover, lactation is generally considered to be a mechanism that is regulated by the hormone of the anterior pituitary gland (9), and the human female breasts show the first signs of glandular activity (i.e., nipple tingling, numbness, enlargement, serous secretion) just at the period when the AP-L hormone is in the urine in increasingly greater amounts (1). In view of these facts, it was decided to examine milk of pregnant cows for the presence of AP-L hormone.

EXPERIMENTAL PROCEDURE

Two types of milk were employed: one, mixed milk (approximately 50 per cent of the cattle which furnish the milk are pregnant [10]), and the other, a single milk, obtained from one pregnant cow at the 6-7th month of gestation, which was bottled, labeled and kept separate from the mixed milk.* The milk was delivered daily for five days, and consisted of samples of the previous day's milking. The milk was not pasteurized, as

*We are grateful to Walker-Gordon Laboratories, Plainsboro, N. J., for all the milk used in the experiment.

it is claimed that heating causes the destruction of the AP-L hormone (11). In every instance skimmed milk was used, since the AP-L hormone is water soluble, and would not be found in the fatty layer (12). The milk was then shaken with ether in a separatory funnel to remove the presence of any ovarian hormone and other impurities that might be present (13). In the case of the "single" milk, the cream, untreated with ether, was also tested for AP-L hormone.

Daily subcutaneous injections of 0.3 cc. each of (a) mixed fat-free milk, (b) fat-free milk from one pregnant cow, and (c) untreated cream from the pregnant cow's milk, were given to immature female white mice 17 days old. As controls, 0.3 cc. human pregnancy urine was injected daily into a fourth group of mice. Six animals were used for each material tested. Six animals were also kept without injection, as a second group of controls. The experimental animals were given daily injections for four days and all the animals were killed on the fifth day by pressure on the spinal column between the occiput and the cervical vertebrae, thus inducing instantaneous death. A gross examination was made of the ovaries and genitalia *in situ* immediately upon opening the peritoneal cavity, and fixed *in situ* with formalin. The ovaries were then removed and the extraneous tissue was cut away. They were then stained *in toto* with borax carmine stain, and later imbedded and sectioned according to the usual routine. Microscopic serial sections of the ovaries of all the animals were made and were carefully studied.

RESULTS

The ovaries of all of the non-injected control animals were pale and clear, showing no evidence of corpora lutea. The group of animals injected with the known positive human pregnancy urine all showed the gross characteristic signs of the Aschheim-Zondek reaction—swelling, turgescence, increase in size of the ovaries, and blood-point hemorrhages. Microscopically they also showed corpora lutea and increased congestion. The groups of mice injected with the mixed milk and the pregnant cow's milk (both fat-free and cream) showed no positive reaction either grossly or microscopically, and were no different from the non-injected group. The entire experiment was repeated one week later with the following slight change in technique. Allen's modification of Bouin's solution (B-15) was used as a fixative for the ovaries *in situ* (14). The ovaries were not stained *in toto* with borax carmine, but were imbedded, sectioned, and finally stained with hematoxylin and eosin. The histological findings of the second experiment were the same as the first.

DISCUSSION

These experiments indicate that in ordinary mixed cows' milk and in single specimens of a pregnant cow's milk, there is either no anterior pituitary-like hormone or too small an amount to give the characteristic Aschheim-Zondek reaction. As a further test, experiments are now being

made on milk concentrated according to the methods used in making urine extracts, with certain necessary modifications (15) (16) (17) (18) (19) (20).

Some of the questions which arise in connection with this work are: Is the anterior pituitary-like hormone of the human non-existent in the cow? If so, does the cow possess a different hormone to achieve the same physiological result? Is there any other channel of body excretion in the cow through which the anterior pituitary-like hormone may escape and hence be overlooked?

SUMMARY

Milk from pregnant cows tested on mice by the Aschheim-Zondek method gave no evidence that the mammary secretion is the path of elimination of the anterior pituitary-like hormone in this animal.

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IDIOPATHIC HYPOPARATHYROIDISM AND TETANY IN THE FOWL*

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Tetany has been noted in several species of mammals, but, so far as can be ascertained, no account of tetany in birds has hitherto been published. This paper reports a case of tetany in the fowl which apparently resulted from temporary subnormal functioning of the parathyroid glands.

A pullet hatched on May 4, 1933, from a cross of S. C. White Leghorn ♂ x Rhode Island Red ♀, began laying on October 27, 1933, at the age of 175 days, her weight then being 2,220 grams. In the 16 days up to November 12 she laid in trapnests a total of 11 eggs, which was a normal rate of egg production. On November 13 this fowl was apparently normal in every respect when the flock was fed at 7:30 A.M., and also at 9:00 A.M., when the birds were given their daily supply of skimmed milk. At 10:15 A.M. she was found prostrate with the legs paralyzed and both wings braced against the floor. When picked up the wings beat spasmodically, but the legs remained paralyzed. When examined at 3:30 P.M. she again underwent uncontrollable convulsive movements of the wings, quite different from the usual flappings made by a captured bird attempting to escape. These were accompanied by dyspnea and by evacuation of the ureters and intestine. The legs remained paralyzed. The convulsions subsided in less than two minutes, but there was no improvement with respect to the paralysis.

Partial or complete paralysis of the fowl may result from several different causes, chief among which are: (1) *neurolymphomatosis gallinarum*, or so-called "fowl paralysis," a practically incurable disease usually accompanied by lesions in the sciatic nerve, (2) polyneuritis resulting from deficiency of vitamin B, and (3) exhaustion of the bird's storage of vitamin D. None of these conditions seemed to be indicated in this case. The flock to which the pullet belonged had been receiving a normal laying ration consisting of wheat and yellow corn (equal parts) fed night and morning, and a hopper-fed mash containing, among the other usual ingredients, wheat germ meal, cod liver oil and 2 per cent of ground limestone. The birds were also given both skimmed milk and ground limestone (96 per cent $CaCO_3$) *ad libitum*. With this ration it seemed doubtful that there could be any deficiency of vitamins, especially so early in the laying period. Nor did the symptoms resemble those of neurolymphomatosis.

On the contrary, the uncontrollable, convulsive movements of the wings, associated with paresis, indicated a case of tetany. Tetany, in turn,

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may arise from different causes, but the fact that the bird had just recently begun to lay suggested that in this case it had been induced by the drain of calcium associated with the formation of shells for the eleven eggs laid prior to its onset. If that were so, presumably the condition might be overcome as in mammals by injecting a calcium salt. Accordingly this was done. The complete protocol of the case follows:

November 13, 4:00 P.M.—Tetanic spasms, paresis. Blood calcium 9.47 mg. Ten cc. calcium gluconate (20 per cent solution) injected intraperitoneally.

November 14, 11:00 A.M.—No change. Five cc. calcium gluconate given intravenously.

November 15-16—Condition worse, no tetanic spasms, complete paralysis.

November 17, 4:00 P.M.—Comatose. Five-tenths cc. Parathormone (Lilly) given intramuscularly.

November 18, 9:30 A.M.—Marked improvement; prostrate but feeds and drinks and attempts to rise. Weight 1949 gm. Twenty-five-hundredths cc. Parathormone injected intramuscularly.

November 20—Improvement; bird walks, but stiffly.

November 21—Improvement; blood calcium 11.51 mg.

December 5—Laying resumed.

December 6—Weight 2201 gm.; blood calcium 19.18 mg.

February 2—Weight 2393 gm.; has laid 48 eggs in 60 days since December 4. Blood calcium 21.46 mg.

INTERPRETATION

Some points in connection with this case require explanation and interpretation. The level of 9.47 mg. Ca in the blood on the day of onset of tetany is only from one-third to one-half of the normal level for a laying hen. It has been shown by Hughes, Titus and Smits (1927), Macowan (1932) and others that while immature fowls, non-laying females, cocks and capons usually have from 12 to 18 mg. Ca per 100 cc. of blood serum or plasma, hens actively laying have levels ranging from 17 to 35 mg. Similar fluctuations were earlier noted in ring-doves and pigeons by Riddle and Reinhart (1926), although in those species the levels were somewhat lower at all stages. Since the fowl with tetany had laid on the day prior to her collapse, her blood calcium should have been about 20 mg. or more.

The output of calcium in the 11 eggs laid before the onset of tetany, assuming that each of them weighed 48 to 50 grams and contained about 1.8 grams Ca, would be approximately 20 grams. This indicates a rather high rate of calcium metabolism for those 16 days, but one not in any way approaching the limits of the species, as hens have been known to lay daily for over one hundred consecutive days. Ordinarily the calcium loss is recovered from the feed, and one's first interpretation of this case would be that the ration was too low in calcium to permit such recovery. Apart from the fact that milk and ground limestone were constantly available

and that the mash contained 2 per cent of ground limestone, as well as calcium in its meat-scraps and alfalfa meal, this interpretation is shown to be erroneous by the fact that the same ration was satisfactory for over 200 other laying pullets which had been receiving it for a period of two months, and among which there occurred no similar cases. Moreover, after the recovery of the bird in question and an experimental period in isolation, she was returned to the flock, where she received the same ration and laid exceptionally well for a period of six months.

If the hypocalcaemia resulted merely from greater calcium loss than calcium intake, one would expect it to have been overcome, as are similar cases in mammals, as soon as the blood calcium was raised to a normal level. The 10 cc. of calcium gluconate injected intraperitoneally, though ineffectual, was approximately ten times the dose in proportion to body weight that effects complete recovery when given subcutaneously in cases of bovine parturient paresis (250 cc. for a cow of about 1000 pounds). The amount of this intraperitoneal dose absorbed by the blood is unknown, but, even if none of it whatever were utilized, the 5 cc. given intravenously on November 14 was enough to raise the blood calcium to an abnormally high level. The writers have been unable to find any determinations of blood volume in the fowl, but, from the figures for several species of mammals, it seems unlikely that the fowl's blood volume would exceed 7 cc. per 100 grams of body weight. On this basis, and assuming the bird to weigh about the same as when she first laid (2,220 grams), the 5 cc. of 20 per cent calcium gluconate [$Ca(C_6H_{11}O_7)_2$] given intravenously would have added about 93 mg. Ca to about 155 cc. of blood. This is approximately three times as much as was necessary to restore the blood calcium to the normal level for a laying hen.

In spite of this there was no improvement and when the first injection of Parathormone was given, 106 hours after the onset of tetany, the bird was on the point of death. The resultant recovery was phenomenal. Eighteen days later laying was resumed and in 176 days from December 5 to May 31, the hen laid 108 eggs. After the original treatments, no further supply of Parathormone was needed to maintain the bird in perfect health. The eggs laid subsequently attained a maximum weight of over 60 grams. The shells were all examined and weighed up to February 2, and in that period they were normal in every respect.

To the writers the best interpretation of the evidence is that the bird suffered from a temporary subnormal functioning of the parathyroid gland at a time when the demand for calcium was particularly heavy. As a result the blood calcium was lowered so much that tetany ensued.

The difficulty is to explain why recovery was not effected when large amounts of calcium gluconate were injected into the blood stream. Calcium therapy is ordinarily successful in cases of tetany, and Lieberman and Szurek (1931) have found the subcutaneous administration of calcium gluconate effective in overcoming tetany even in dogs apparently completely parathyroidectomized. It is now a standard treatment for parturient

paresis in cattle. In this case calcium therapy was entirely ineffectual, but treatment with parathyroid hormone produced immediate results. It seems probable, therefore, that the original hypocalcaemia and tetany resulted, not from any unusual drain of calcium, but from temporary idiopathic hypoparathyroidism. Instances of this sort are apparently rather rare in man, but Davidson (1925), Liu (1928) and Albright and Ellsworth (1929) have reported chronic cases effectively treated with parathyroid extract.

The effectiveness of Parathormone, when injection of calcium had failed, suggests that its function is not so much to raise the level of total blood calcium as to convert some of the calcium into a form, or forms, indispensable to the bird. Heller, Paul and Thompson (1934) found that the rise in blood calcium associated with egg production results chiefly from a marked increase in the adsorbable non-filterable fraction while the adsorbable filterable calcium decreases and the ionized remainder stays fairly constant. It seems probable that the rôle of the parathyroid hormone in avian reproduction may be to increase the adsorbable non-filterable calcium.

DISCUSSION

In view of the amount of calcium metabolized by the fowl in the formation of egg shells, it is remarkable that cases of hypocalcaemia and tetany are not more common in this species. So far as the writers can ascertain, no such case has been reported in the fowl or in any other bird, except for the not uncommon occurrence of paresis (without tetany) in flocks long maintained in egg production without an adequate supply of vitamin D. Such instances are usually found in February or March in confined flocks not receiving cod liver oil, or its equivalent, and are easily cured by supplying vitamin D. The limits of calcium tolerance (up to 35 mg. per 100 cc. blood serum) are much higher in birds than in mammals and the ability of birds to mobilize calcium very rapidly has been amply demonstrated (Riddle and Reinhart, 1926). Presumably these capacities have been evolved as necessary corollaries to the avian type of reproduction. This same facility in metabolizing calcium may be adequate protection against hypocalcaemia except when the available calcium or vitamin D is restricted (in which cases ovulation is retarded and eventually ceases), or, as in the present case, when the parathyroids fail to function normally.

The present case extends somewhat the previous knowledge of the effects of administering parathyroid extracts to birds. Collip (1931) was unable to demonstrate any effect of the hormone after injection in non-laying hens. Similarly, Macowan (1932) found Parathormone to have no effect on the blood calcium in moulting fowls, even when injected in doses as large as 1 cc. Smaller doses did cause a rise in the blood calcium of immature females. The writers' case demonstrates a marked response to Parathormone in a bird in which the parathyroids were apparently not functioning normally.

SUMMARY

After laying 11 eggs, a young pullet was afflicted with tetany and paresis, and found to have only 9.47 mg. Ca per 100 cc. blood plasma, when 20 mg. would have been normal.

Liberal doses of calcium gluconate given intraperitoneally and intravenously had no effect and the paresis persisted, with tetany succeeded by coma.

Treatment with Parathormone (Lilly) given on the fifth day resulted in complete recovery. Blood calcium was raised, egg production was resumed and the bird laid exceptionally well for a period of six months thereafter, maintaining 21.46 mg. blood calcium 77 days after administration of Parathormone.

The case was diagnosed as one of hypocalcaemia and tetany resulting from temporary idiopathic hypoparathyroidism. It is apparently the first case of tetany reported in birds. It is suggested that the rarity of hypocalcaemia and tetany in birds may result from special facilities for metabolizing calcium evolved as necessary functions in the avian type of reproduction, and that the rôle of the parathyroid hormone in that process is to increase the non-filterable adsorbable calcium in the blood.

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THE ENDOCRINAL ORIGIN OF PRIMARY DYSMENORRHOEA AND ITS HORMONAL TREATMENT

A PRELIMINARY REPORT

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Primary dysmenorrhoea is truly spoken of as one of the "bugbears" of gynecology. Many theories as to its etiology, mechanical obstruction, acute uterine anteflexion, hypoplasia of the uterus, psychogenic and constitutional disturbances, have been advanced, refuted and discarded. The most important cause of primary dysmenorrhoea, and the one dealt with extensively in this paper, is endocrine imbalance.

Undoubtedly the immediate and actual cause of the pelvic cramps and pain is the spasmodic contraction of the uterine muscle; the very nature of the pain suggests this as the cause. Only recently has uterine contractility been investigated; Knaus in Austria and Reynolds in this country have been the main investigators. The latter studied the uterine contractions of experimental animals in the unanaesthetized condition. The advantage of this is obvious in view of the recognized influence of practically all forms of anaesthesia on uterine motility.

Through an abdominal incision in rabbits, Reynolds cut across the vagina below the cervix. The vaginal cuff was then turned downward, and the cervical end was brought through the abdominal incision and sutured in situ, thus creating an abdominal uterine fistula. A small balloon was introduced into the uterus and connected to an air-water manometer and kymograph. By this arrangement the action of drugs and hormones on uterine contractility could easily be studied.

Reynolds' investigations have shown that the oestrous phase in the non-pregnant rabbit is associated with marked uterine activity, while anoestrus is characterized by only feeble motility, if any at all. Translated to the human problem, uterine rhythmic contractions become increasingly more active as the graafian follicle develops. After rupture of the follicle and corpus luteum formation, the uterine contractions are inhibited, so that during the last half of the menstrual cycle, the progestin phase, the uterine muscle is relatively quiescent. Castration produces complete quiescence of the uterine muscle. Activity can be restored by substitutional therapy with the follicular hormone, again showing that this hormone stimulates uterine activity. Experiments with other endocrine preparations show that the follicular hormone alone possesses this property.

In these castrated animals whose uteri have been whipped into activity by the follicular hormone, the administration of the hormone derived from pregnancy urine, like that of progestin, inhibits uterine motility. This

observation is noted even in normal rabbits when the pregnancy urine hormone is given in such small doses that it does not cause any ovarian change. Hence it may be concluded that the action of the hormone of pregnancy urine is a direct one on uterine muscle. The action of this hormone, however, is common to other animals, since it produces uterine activity in the mouse, rat, guinea-pig, ferret, monkey and other animals.

Knaus in Austria studied uterine motility in human subjects by a technique based on the responses to posterior pituitary extract (pituitrin) exhibited by the uterine muscle at different phases of the cycle. He found the uterus responsive to stimulation by pituitrin in the first half of the menstrual cycle. After ovulation the uterus is refractory to pituitrin and is quiescent.

The pain in primary dysmenorrhoea, as noted, is characteristically of a spasmodic, colicky nature: it begins a day or two before the onset of the menstrual flow, theoretically the time when the lutein body begins to degenerate, and when the follicular hormone stimulation again comes into its own. The height of the corpus luteum activity is several days before the onset of the menstrual flow, not at the time of flow. This is well illustrated by excision of the corpus luteum; the menstrual flow occurs 24 to 48 hours later. The pain of primary dysmenorrhoea can be explained by the withdrawal of the progestin influence, which results in whipping into marked uterine activity, by the action of the follicular hormone, a uterus which has been lying in a quiescent state for 10 days to two weeks. In fact, according to Knaus' work on human subjects, a day or so before the onset of menstruation is the period of maximum uterine muscular activity.

That this hormonal sequence occurs in all women is undoubtedly true, but only a comparatively few women suffer with dysmenorrhoea. Two explanations can be offered: (1) a heightened sensitivity to the pain stimuli because of psychogenic or constitutional factors, and (2) a real endocrine imbalance acting on uterine motility. This imbalance may be a quantitative factor, marked uterine contractions resulting from excessively large amounts of the follicular hormone over progestin; or it may be to a chronological factor, the follicular stimulation coming on too abruptly, not a gradual stimulation, after the withdrawal of the corpus luteum influence.

The aim of treatment in this condition is either to counterbalance the excess of the follicular hormone by substituting additional corpus luteum influence, or to withdraw the corpus luteum influence slowly, so that the follicular hormone stimulation of the uterus will not be precipitated, but will appear gradually.

The corpus luteum hormone, progestin, is not available for commercial use, but the luteinizing factor obtained from the urine of pregnant women is prepared in an aqueous glycerin solution by several of the large drug houses. The preparation used in this series of cases is termed Follutein, and was made available for our use by the kindness of Dr. J. F. Anderson of E. R. Squibbs & Sons. One cc. equals 250 R.U.

The method of treatment employed was to inject intramuscularly and daily, from 3 to 4 days previous to the expected flow and 1 to 2 days during the flow, 250 R.U. (1 cc.) of Follutein. If the primary dysmenorrhoea was associated with uterine hypoplasia, the ovarian follicular hormone, Amniotin (E. R. Squibbs & Sons) was administered also in daily 1 cc. doses (50 R.U.) from the time of cessation of the flow until the mid-interval phase of the cycle, the time of ovulation. The rationale of this therapy is that the ovarian follicular hormone stimulates uterine growth. In theory it should in time convert the hypoplastic uterus into a normal one. In this connection Clauberg has published skiagrams showing by means of lipoidol injections the greatly increased size of the cavity of the uterus

TABLE I

Age	Menarche	Duration of Symptoms	Fertility	No. of Injections	Duration of Observation	Subjective Results	Objective Results
		Years			Mos.		
22	16	6	0	10	5	No pain.	2 periods without treatment, asymptomatic.
16	14	2	0	10	7	No pain.	5 periods without treatment.
14	13	1	0	17	5	Happy over result.	1 period without treatment, asymptomatic.
16	13	2	0	23	6	Not much better.	No improvement.
22	15	5	0	15	5	Still has cramps.	Slight improvement.
20	18	2	0	10	9	Entirely cured.	7 periods without treatment, asymptomatic.
18	12	4	0	15	4	Severe cramps.	No improvement.
21	11	8	0	15	12	Does not suffer at all any more.	10 periods without treatment, asymptomatic.
17	13	2	0	15	6	Cured.	2 periods without treatment, asymptomatic.
19	15	2	0	15	5	Periods painless.	1 period without treatment, asymptomatic.
20	11	9	1	10	6	Feels fine.	3 periods without treatment, asymptomatic.
20	16	4	0	12	9	Much better.	5 periods without treatment, asymptomatic.
22	14	8	0	12	8	Severe pain.	No improvement.
19	12	4	0	10	11	Pain half as bad.	7 periods without treatment, asymptomatic.
21	11	10	0	14	10	Pain relieved.	6 periods without treatment, asymptomatic.
19	13	6	0	12	9	Pain relieved.	4 periods without treatment, asymptomatic.
16	13	3	0	16	9	Pain greatly relieved.	5 periods without treatment, asymptomatic.

in a castrated woman after large injections of the ovarian follicular hormone. In cases in which uterine hypoplasia was not present, no treatment was given until just before the next expected period.

Seventeen patients suffering from primary dysmenorrhoea were so treated; in 13 cases the results were very satisfactory. In one only slight relief of pain was noted; in the other three patients the dysmenorrhoea continued in its severe form.

A rather important observation is the youth of the treated patients; the oldest was 22 years. Whether or not this method of therapy would be beneficial to older women with so-called primary dysmenorrhoea is a speculation.

The only untoward symptom noted was an occasional local reaction at the site of the injections. This unpleasant symptom could be somewhat alleviated by lessening the dosage given or by diluting the amount of fluid injected with an equal volume of sterile normal saline solution of 0.5 per cent novocain solution. Occasionally the menstrual periods were delayed 3 or 4 days. This is easily explainable, since it is generally accepted that progestin is an inhibitor of menstruation.

Hormonal therapy will be of value in the treatment of primary dysmenorrhoea if permanent benefit results from its use. The time which has elapsed since the cessation of hormonal treatment in this series of cases has been too short to determine whether or not the relief of pain obtained in some patients will be permanent. In general we carried each patient over three series of injections, that is over a three-month course of treatment at monthly intervals, and then stopped all hormonal therapy.

SUMMARY

The most recent explanation of primary dysmenorrhoea is an endocrine imbalance. Reynolds has shown that uterine rhythmic contractions become increasingly more active as the graafian follicle develops. After rupture of the follicle and corpus luteum formation, the uterine contractions are inhibited. The pain of primary dysmenorrhoea can be explained by the withdrawal of the progestin influence, which results in whipping into marked activity, by the action of the follicular hormone, a uterus which has been lying in a quiescent state for ten days to two weeks.

The rationale of treatment in this condition is to counterbalance the follicular hormone activity by administrating the luteinizing principle found in the urine of pregnant women. The method of treatment employed was to inject intramuscularly and daily, from three to four days previous to the expected flow, and one to two days during the flow, 250 R.U. (1 cc.) of Follutein. Thirteen of seventeen patients so treated for dysmenorrhoea experienced relief.

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THE CORPUS LUTEUM OF PREGNANCY IN RELATION TO THE ANTERIOR PITUITARY GLAND*

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The corpus luteum produces two hormones in varying amounts, oeostrin and progestin. The latter has been found to be important for the embedding of the ovum and for the preservation of the embryo during its early life. Whether the presence of the corpus luteum is necessary for the anterior pituitary gland to exercise its hormonal influence during early pregnancy, has not, as far as I know, been subjected to clinical observation. The following case, in which the Aschheim-Zondek test (Friedman modification) was repeated thirteen times after the removal of a twisted ovarian dermoid and a corpus luteum cyst complicating pregnancy, afforded an opportunity of noting whether the pregnancy reaction persists in the absence of the corpus luteum.

Mrs. M. T., a private patient of Dr. Richard Hoffmann, was operated upon, June 12, 1934. Her last menstrual period began April 16. Previously symptom-free, she was suddenly seized with severe pain in the left lower abdominal quadrant and vomiting two days before her admission to the hospital. A large globular mass was found in the left half of the pelvis; the uterus was enlarged and soft. At operation there was a moderate amount of sero-sanguinous fluid in the pelvis. The uterus was found to be enlarged to the size of a seven-weeks' gravidity. There was a twisted dermoid ovarian cyst on the left side, which was removed. The right ovary contained a smaller cyst, which was also removed. The latter, upon histological examination, was found to contain the corpus luteum of pregnancy but no dermoid particles.

It was thought worth while to determine whether the anterior pituitary gland, in the absence of the corpus luteum, still secreted the hormone in the urine upon which the Aschheim-Zondek reaction depends. The Aschheim-Zondek test (Friedman modification) was therefore performed the day following operation and repeated at three-day intervals. The pregnancy test remained positive for one month after the primary operation, the last urine being examined July 13. The patient, however, began to bleed June 25, thirteen days after the operation. The bleeding, at first like a menstrual flow, became more profuse and continued until July 16, when it was found necessary to resort to curettage. The Friedman test three days later was still positive, and only on July 21, thirty-nine days after the removal of the corpus luteum, did the test become negative.

The removal of the corpus luteum may have been responsible for the interruption of the pregnancy in this case. This cause and effect rela-

*I am indebted to Dr. Richard H. Hoffmann for the suggestion of this investigation.

tionship is not absolutely established for the human, although it appears to be in animals. The results in this case, however, show that the corpus luteum apparently plays no part in the phenomenon of the pregnancy reaction obtained by the Aschheim-Zondek test (Friedman modification).

SUMMARY

The case is reported of a woman from whom in the seventh week of pregnancy a dermoid ovarian cyst on one side and a corpus luteum of pregnancy on the other were removed surgically. The pregnancy was terminated by curettage five weeks later because of profuse bleeding. The Aschheim-Zondek test, repeated at three-day intervals, was found to be positive on each occasion for 39 days after the removal of the corpus luteum. It is suggested that deprivation of the corpus luteum may have been instrumental in causing the termination of the pregnancy but obviously did not interfere with the hormone production determining the Aschheim-Zondek test.

A STUDY OF THE EFFECTS OF INTERMEDIN AND INJURY OF THE HYPOPHYSIS ON TRAUMATIC CORIAL MELANOPHORES IN GOLDFISHES

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A red color reaction in Phoxinus laevis, following injections of intermedin, was first described by Zondek and Krohn (1932). Cutaneous erythrophores, and to a less extent melanophores, expanded soon after hypodermic injections of intermedin, giving rise to a diffuse brilliant redness, similar to what is known as the Hochzeitskleid, normally present at the time of mating. This color reaction in Phoxinus laevis was regarded as a specific test for intermedin. Later Ferguson (1933) found the substance intermedin, as tested by the red eruption in Phoxinus laevis, present in the blood and in tumor tissue of certain patients with melanotic growths.

Several years ago, Smith (1931) noted in goldfishes subjected to trauma, a transient eruption of corial melanophores. This eruption of black pigmented cells began at the site of injury approximately on the fourth day and became intensified in the next few days. Depigmentation was usually accomplished in the course of three weeks, the melanophores undergoing degeneration. Fishes kept in heated water showed an acceleration of the eruption and depigmentation, while conditions of cold retarded or even inhibited the eruption. Darkness had no effect. Subsequently it was shown that exposure to x-rays elicited a similar melanophore eruption and depigmentation (Smith, 1932). It was pointed out that such corial melanophores, the result of trauma or x-rays, were peri-vascular in distribution, and not necessarily connected with the nervous system as are the ordinary melanophores found in the skin of fishes which give rise to patterns and color changes as a result of nervous stimuli as shown by Pouchet (1876), von Frisch (1911), Wyman (1924), and others.

Ferguson continuing his investigations (1934) found that Intermedin could be recovered not only from the blood and tissue of melanotic tumors but from blood and tissue of other tumors as well. Appreciable amounts were found chiefly in that group of tumors presumed to be of neurogenic origin or in tumors in which there was an associated disturbance of pituitary physiology. This lead us to suppose that the effect of the hormone was chiefly on the peripheral nevus cell, in contradistinction to the corial melanophore.

It became of interest, therefore, to learn if intermedin, a hormonal substance on the one hand, found in human melanotic tumors and blood of persons suffering with melanotic tumors, and, on the other hand, possess-

ing properties of expanding pigment cells of Phoxinus, might affect the cycle of corial melanophores as seen in goldfishes after injury. To this end a number of experiments were carried out on goldfishes, traumatized at definite cutaneous points, which either before or after injury received injections of assayed doses of intermedin. These experiments were supplemented by a short series of studies in which hypophysectomy or damage to hypophysis was instituted in goldfishes to learn the effect also of these measures upon the above mentioned eruption of melanophores following cutaneous injury.

In neither group of experiments (a) in which goldfishes were subjected to trauma plus injections of intermedin, or (b) in which injury or removal of hypophysis was performed, were the effects on the eruption or depigmentation of wound melanophores noted. These experiments suggest, therefore, that the hypophysis probably does not exercise control over the formation and later degeneration of black pigmented cells known to occur in the corium of traumatized goldfishes, but rather strengthens the belief that this localized wound pigmentation is the result of a temporary activity of pigment-producing cells linked locally with the growth of blood capillaries in the repair of tissue.

This finding is regarded of interest in as much as in amphibians, as the result of studies of a number of investigators, including Smith (1916), Allen (1916), Swingle (1921), Atwell (1919), Blount (1932), it has been demonstrated that the hypophysis is active not alone in the production of substances which affect melanophore expansion, but that it also acts with the thyroid gland in the control of general pigmentation.

EXPERIMENTAL STUDIES

(A), *Trauma Plus Injections of Intermedin.** 3/20/34. Ten goldfishes, 8 cm. in length, were traumatized by means of an artery clamp applied transversely to the base of the tail and the right operculum for fifteen seconds. Immediately after, each fish was injected with 0.05 cc. of sheep's intermedin assayed at 4000 units per 1 cc. Every other day thereafter the fishes received a similar dose, and this was continued up to ten doses. Four control fishes of the same size received similar trauma, but were not injected with intermedin. All fishes were kept in tanks of water with a temperature of 70° F., supplied with a current of air.

3/25/34 (five days after injury). At crushed points of tail and operculum in all fishes, including controls, there was found well-developed eruptions of corial melanophores seen by the dissecting microscope. These points of localized wound pigmentation became intensified in the course of the next two or three days, and then began to disappear by degeneration of individual pigment cells. Depigmentation in all fishes, including controls, was completed by 4/20/34, or one month after injury. In all, 26 goldfishes were experimented on in four different groups with injections of intermedin. Although the dosage of intermedin and the timing of the dosage was varied somewhat for each group of experiments, the behavior of the pigmental eruptions remained within the range of normal.

*The Intermedin preparation used in the above extracts was prepared according to the technic used by Zondek and Krohn (1932). Our solutions assayed approximately 2000 fish units per c.c.

(B). *Injury To and Removal of the Hypophysis of Goldfishes Plus Experimental Crushing of Tail and Operculum.* 3/22/34. Five goldfishes 8 cm. in length were operated on for removal of the hypophysis by the following method:

The goldfish was anesthetized in chloretone solution, wrapped in a damp towel. The head was left exposed and held in position on the stage of a dissecting microscope. With iridectomy scissors and fine lances the right eye was removed. Since the hypophysis lies immediately behind the decussation of the optic nerves, the trunk of the right nerve was used as a land mark for the pituitary. By following along this nerve it was possible to reach the immediate vicinity of the gland. This involved considerable trauma to the interorbital plates and invariably ruptured an artery which considerably obscured the field. Somewhat blind groping was enforced by this circumstance. By forceps and blunt probes the space occupied by the pituitary was traumatized. Therefore, an effort was made in each case to excavate as much material from this region as possible. Too much trauma in this region must be avoided to prevent killing of the animal. It was found to be unnecessary to close the wound in any way and the fish was returned to its normal environment as promptly as possible. Following a recovery period of several hours, the operated animals showed no obvious disturbance in their normal behavior.

On the same day the tail and the right operculum of each fish was crushed by means of an artery clamp for fifteen seconds. Five days later eruptions of melanophores developed at the crushed points and at the operative wound in the head region in all fishes. Depigmentation was completed twenty-six days after operation (4/18/34). Histological examination of decalcified heads showed one fish with complete removal of the hypophysis and four fishes with some detached and degenerated hypophyseal tissue in the cranial cavity. The course of melanophore eruptions and depigmentation in all five fishes were within the range of normal limits for fishes kept in water at temperature of 70° F.

In all, 17 goldfishes survived the necessary experimental period after operations on the hypophysis. In only one was a complete removal accomplished as verified by subsequent histological examination of the brain and cranial cavity. In all of them severe damage to the stalk, tuber, or the hypothalamus was found. In no instance was there found any normal pituitary substance. Obviously the experiments do not preclude the possibility of some chemical agent escaping from the remains of the gland cells into the general circulation. In the one instance of completely successful removal the results were not in any way different from the cases in which there was severe damage to the pituitary. This suggests that the hypophyseal mechanism bears no significant relationship to the production of the black pigment cells resulting from trauma in the goldfish.

SUMMARY

An attempt was made to influence the course and intensity of wound pigmentation in the goldfish by the injection of intermedin, a hormonal substance found to exist in the blood and tumor tissue of certain patients with melanotic growths. No effect was noted on the cycle of corial melanophores in 26 traumatized goldfishes. When the hypophysis was removed or injured in 17 goldfishes, there was also no effect on development of corial melanophores or their subsequent degeneration. These experiments

suggest that the hypophyseal mechanism has no significant relationship to the cycle of corial pigmentation and subsequent depigmentation in goldfishes following trauma.

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EFFECTS OF PREGNANCY URINE ADMINISTRATION TO FEMALE SWINE

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Sterilized pregnancy urine was administered to female swine to determine if a depressing effect upon the sexual activity of these animals could be obtained and also to determine if such treatment would be feasible for use in the fattening period before marketing.

Preliminary experiments were made upon male and female rabbits. Prolonged administration of prolan or sterilized pregnancy urine (urine taken in third month of pregnancy) were made and it was found that the majority of the animals showed a lowering of sexual activity and a tendency to adiposity. Some of the animals showed complete atrophy of the sex glands.

The experiments with pregnancy urine were then made on 40 swine, aged 4 to 5 months. These selected swine were weighed, numbered and divided into a control group and an experimental group, each containing 20 animals. Both groups were kept in adjacent pens in a common yard and were fed an equal food ration which consisted of dining room leavings without any added concentrates. The animals were weighed once a month. The treatment consisted of 10 injections made every second or third day. The injections were made subcutaneously behind the ear and a single dose ranged from 10 to 20 cc. The total amount injected ranged from 175 to 200 cc., corresponding to 875-1000 mouse units of prolan.

After the first two or three injections the treated animals gained weight more rapidly than did the controls. They were excited, lively and showed a greater relish for food. Later they became depressed, apathetic, ate less than the controls and lost weight.

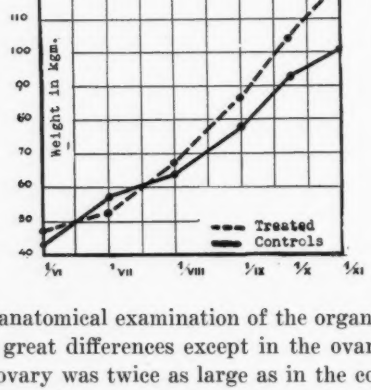
Of the 20 experimental animals only two showed sexual excitement, whereas all the control animals showed typical manifestations of rut. After the injections were stopped the experimental animals showed a quick recovery and by the end of the second month they weighed considerably more than the controls.

The observations were terminated four months after cessation of treatment and one animal (the largest) from each of the two groups was slaughtered.

The monthly weighing of the animals showed that, after the treatment had been discontinued, the experimental group weighed more than the controls and by the end of the fattening period the difference averaged 17.2 kgm. per animal. The results of the periodic weighings are shown in the accompanying curve.

The total weight increase over a five-months period was 55.8 kgm. per animal in the control group and 73.0 kgm. in the experimental animals, a difference of 17.2 kgm. The daily increase in weight was 115 grams greater in the treated group.

After slaughtering it was found that the carcass weight of the experimental animal was greater by 10.67 kgm. than that of the control, the weight of fat was 1.89 kgm. more and the depth of the superficial fat was 1 cm. greater.



The pathologo-anatomical examination of the organs of the slaughtered animals showed no great differences except in the ovaries. In the experimental animal the ovary was twice as large as in the control animal and it showed obvious signs of degeneration in the form of a cystic luteinization. On the surface of the degenerated ovary enlarged cystic-like follicles full of blood could be seen as well as yellow corpuscles the size of peas.

SUMMARY

Prolonged administration of large doses of pregnancy urine (not less than 875 mouse units of prolan) to 20 female swine (4-5 months old) seemed to cause permanent inhibition of sex activity. Degenerative changes were found in the ovaries of a treated animal that was slaughtered and the ovaries were about twice the size of those of the control. The carcass weight, fat weight and depth of superficial fat were greater in the treated animal.

THE EFFECT OF INJECTION OF RESIDUAL OVARIAN EXTRACTS*

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INTRODUCTION

Many studies have been made on whole ovary extracts, and on extracts of the follicular fluid and corpus luteum of the ovary. In recent years the residual ovary (portion remaining after removal of the follicular fluid and corpus luteum) has received more attention as a possible source of the sex hormone. There is much unanimity of opinion on the observation that the male sex hormone is elaborated by the interstitial cells of the testes. There is no such agreement as to the function of the interstitial cells of the ovaries. These cells might elaborate the female sex hormone, or its precursor, some other hormone, or nothing at all.

Graves (1) points out that Simon, Jolley and Marshall have shown that in ovarian grafts the follicles degenerate while the interstitial cells remain unchanged. He concludes then that these cells must have secretory function. Lipschütz (2) shows that x-ray treatment does not injure the interstitial cells as much as the remainder of the ovary, and while sterility results, secondary sex characteristics are not affected. These results, he explains, were caused by an increase in the interstitial cells derived from the ovary. Also, since grafts are in the main interstitial cells, and are able to perform the internal secretory function of the ovary, these cells must be the source of the female sex hormone. Jaffe and Marine (3) doubt that these cells of the ovary are concerned in the production of the hormone. Moore and Price (4) have concluded that since the pituitary is less active in the pregnant animal than in the non-pregnant female, the urinal-gonadal-stimulating hormone is not of hypophyseal origin but perhaps arises somewhere in the ovarian stroma. Kingsbury (5), Wilkerson (6), and others can find no glandular tissue in the ovarian stroma. They have decided that there is no ovarian secretion from this source. D'Amour, D'Amour and Gustavson (7) have found the estrin content of the follicular fluid to be one rat unit per cc., and of the corpus luteum to be 65 rat units per kilogram of material. They report no studies upon the ovarian stroma.

It can then be seen that although some workers point to the ovarian stroma as the source of the sex hormone, others are as firmly convinced that there is no reason to expect the interstitial cells to be the source of the female sex hormone. However, no exhaustive research has been reported as the interest in this field has been concerned largely on isolation of this

*Contribution No. 187, Department of Chemistry.

hormone from the urine of pregnant females, and to a study of its composition and structure.

Since one of us (H. W. M.) had prepared an extract from the ovarian stroma containing a minimum of follicular fluid and corpus luteum, it was decided to test such an extract physiologically and histologically on immature albino rats and mice.

PREPARATION OF EXTRACT

Fresh sow ovaries, packed in ice, were obtained from the Wilson Packing Company. The follicular fluid was aspirated from the ovaries by means of suction and a hypodermic needle in the usual manner. Corpus luteum bodies were dissected out, leaving what is referred to in this paper

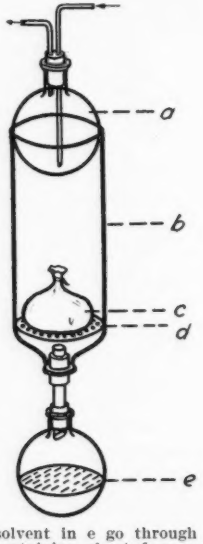

Figure 1. The vapors from the solvent in e go through the opening in the porcelain plate, d, through the muslin sack, c, containing about four pounds of ground ovaries. The vapors are condensed on a and in the sack, and return to the receiver, e. The contents of the sack should be kneaded once or twice to prevent caking. This type of continuous extractor is inexpensive, permits large samples to be extracted efficiently, and permits frequent changing of solvent to avoid too long heating of the extract. The extract is free of extraneous material and does not need to be clarified.

as "residual ovaries," or "interstitial tissue of the ovaries." The residual ovaries were placed in four volumes of 95 per cent alcohol until all the ovaries from thirty-five pounds of tissue were ready for extraction. They were then ground, and separated from the alcohol by pressing through cheese cloth. About four pounds at a time were placed in a special extractor (Figure 1) and extracted from three to five times with fresh alcohol until the final extract did not show coloration after one hour's extraction. The alcoholic extracts were combined and placed in an ice box overnight in order to allow the separation of neutral fats. The alcoholic liquor was removed from the fats by decantation, and the neutral fats were washed

several times with cold alcohol by centrifugation. The combined alcoholic liquors were reduced to a sludge by distillation under reduced pressure. The sludge was taken up in alcohol and again reduced to a sludge. Ether was employed to dissolve this residue, and three volumes of acetone added to remove the phospholipins. The ether-acetone mixture was evaporated under diminished pressure, and the residue dissolved in the least amount of hot alcohol. This was cooled overnight in an ice box, and cholesterol together with some fatty material separated from the alcohol. The alcohol was removed from the solids by filtration, and the alcohol was then distilled under diminished pressure. This left a semi-crystalline, oily-like mass. This residue will be referred to as the "purified extract."

To make a preparation suitable for injection, one gram of the purified extract was dissolved in a small amount of alcohol, 20 cc. of pure olive oil added, and the alcohol removed under reduced pressure. This left a clear solution slightly darker in color than the original olive oil. This gave an equivalent of 14.5 grams of fresh material per cc. of olive oil solution. Preliminary experiments indicated that ¼ cc. of above solution administered subcutaneously daily gave positive results. This dosage was adopted as a routine measure.

EXPERIMENTAL PROCEDURE

A preliminary study of the extract was made on 21-day-old mice. The female mice showed prematurity in three to four days as evidenced by the opening of their vaginas. For convenience, and because of availability, 21-day-old albino rats were used. The experimental animals received a dosage of ¼ cc. per day each, and the litter mate controls received the same amount of pure olive oil. (The former are referred to in the table as "A" and the controls as "B.") General health, physiological activity, sexual development, weight records and, when possible, vaginal smear records were noted as routine procedure.

Other experiments were carried out on animals 49 days old, and 60 days old, older normal and ovariectomized rats, and some young immature male rats. In all, about 175 animals were used.

In the younger growing animals there was a steady gain, and a like gain in weight of both experimental and control males and females.

The effect upon the reproductive system of the 21-day-old females was positive and consistent. The vaginas of the experimentals were open in from seven to ten days. The controls showed no such sexual growth even when they were as old as 46 days while receiving daily dosages of olive oil. Daily vaginal smear records showed that for the most part a normal heat cycle was evident in the experimentals, and continued as long as the injections were continued. (See Table I.) Animals were injected for as long as 25 days after the opening of the vagina. A few of the lot showed a long cycle of from 8-10 days, but they were much in the minority. Most of them, however, showed the normal 5-6 days cycle as normal sexually mature rats show. Normal young fertile males were placed in the pens with some

*TABLE I

EFFECTS OF EXTRACTS ON YOUNG FEMALES

Animal	Day Vagina Opened	Size of Uterus	Day After Vagina Opened on Which Smears Were Made													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
									Sta	ges						
A145	10	17x1.5	1	2	5	1	5	4	..	..	..	..	..	..	..	..
A146	8	20x3	1	2	2	2	4	5	5	1	5	1	5	1	..	..
A147	8	20x3	5	5	5	1	2	5	5	2	4	..	..	..	..	..
A165	12	22x1.5	1	5	5	3	5	2	2	3	5	..	..	..	..	..
A166	10	22x2	5	5	5	5	5	3	5	2	2	2	5	5	1	..
A181	8	17x2	1	2	2	5	1	3	5	5	5	5	5	..	..	..
A182	8	.....	4	1	2	4	2	5	5	5	1	2	..	..	..	..
A184	9	.....	1	4	2	1	2	2	2	1	2	4	..	..	..	..
A186	8	.....	5	2	2	5	2	2	4	5	1	2	..	..	..	..
A192	8	.....	1	2	4	4	5	5	1	5	5	1	..	..	..	..
B133	..	23x .5	Vaginas not open.													
B140	..	17x .8														
B153	..	22x1.9														
B154	..	22x .5														
B163	..	22x .5														
B165	..	22x .5														
B166	..	22x .5														
B171	..	17x .5														
B172	..	22x .5														
B173	..	22x .5														
B182	..	17x .5														

Stage 1—Proestrus—Small and large nucleated epithelial cells.
Stage 2—Early Estrus—Cornified and large epithelial cells.
Stage 3—Typical Estrus—Cornified cells only.
Stage 4—Late Estrus—Some cornified and leucocytes in abundance.
Stage 5—Diestrus—Small epithelial cells and leucocytes in abundance.

of the prematured females, and daily smear records kept. There was no evidence of copulation having taken place either in the smears, or in the condition of the animal afterwards.

A second effect of this extract on the younger females was upon the size of the uteri. The uteri of the experimentals measured from 17-22 mm. in length, and 1.5 to 3 mm. in diameter, while the controls showed the same average length, but only 0.5 to 1 mm. in diameter, averaging 0.6 mm. in diameter. No difference could be noted between the experimental males and controls as to size of testes, or in histological structure.

A third effect noted was on the appearance of the uteri. The controls appeared opaque while the experimentals were to a much greater extent transparent.

A fourth effect noted was in the histological study. While follicular atresia was noted in both experimentals and control ovaries, the former certainly showed more embedded ova than the latter.

An experiment was attempted to show the possible mechanism of the effect. Pituitaries were removed from young rats that had been prematured by the extract, and whose vaginas had just opened. A pituitary was implanted in a rat 21 days of age, until 10 successive implantations were made. The pituitaries were removed from the donor daily and just before implantation. The vaginas opened on the tenth day, and the uteri were enlarged. The controls received a pituitary from an animal of the same age

*Taken from an unpublished M.S. Thesis of one of us (F. G.) and loaned through the courtesy of the Department of Zoology and Dr. G. E. Johnson.

and a litter mate of the donor for the experimentals. The controls remained infantile. This seemed to indicate an active pituitary in the treated animals and that the effect of the extract upon the gonads was through its effect upon the pituitary, and in turn the pituitary excited to greater physiological activity.

Another group of animals was prepared by ovariectomizing some of them, uterectomizing some, and both ovariectomy and uterectomy practiced on some. In all animals the vagina opened in ten days, and the uteri remaining were enlarged and transparent.

Still another group was injected with varying doses of pure theelin incorporated in olive oil to determine the minimum dosage necessary to cause an opening of the vagina in the same time as the purified extract. After several trials 5 rat units was chosen as the proper dosage. Another group then received 5 rat units of theelin and another group, ¼ cc. of the purified extract daily. The vaginas of both groups were open on the ninth day. The animals were autopsied and measurements made on their respective uteri. The uteri of the theelin-treated animals were all less than 1 mm. in diameter, with an average of 0.7 mm., while the uteri of the animals treated with the purified extract were none less than 2 mm. in diameter, and the average greater than two. The length of uteri in both groups was the same. If theelin is one of the controlling forces, it seems to the authors not to be the only hormone involved. To us this points to a pituitary-exciting material from the stroma of the ovary.

Older rats, aged 49 and 60 days, and older females as well, some normal, and some ovariectomized, were treated with the same dosage, and some with double the dosage given the smaller rats. The uteri of these mature animals were between 1½ mm. and 2 mm. in diameter, and from 12-14 mm. in length. After 25 days' treatment, the uteri of the normal rats were 5 mm. or more in diameter, and the uteri of the castrated rats were smaller than the controls (normal rats). The regular heat cycle was not disturbed on any dosage given, and the castrates showed an anoestrous smear throughout. Control periods to determine the heat cycle of the individuals were run before the injections started. The same equivalent dosage from whole ovary extracts caused all animals to go into estrus and remain thus for a period of 3-5 days for one injection of 1 cc. Again this points to some action other than that of theelin.

DISCUSSION OF RESULTS

Precocious development of the genital system of young animals has been noted by many workers, among whom are Allen, Doisy and co-workers, who have used theelin prepared from the urine of pregnant animals. Collip has produced like results with an extract of human placenta. His results have been confirmed by one of us (H. W. M.). Others have produced like results from pituitary extracts, and implants (Smith and Engle, Zondek and Aschheim, G. E. Johnson and his co-workers). The authors of this paper are not convinced that the results with the extract from the

residual ovaries on uteri development and smears, could solely be caused by theelin. Either there is some other substance present that may affect the sexual development through first affecting the pituitary, or the action of extracts from tissue have different or more lasting effects than the pure theelin. Mathematically, at least, according to the results of Gustavson with pure follicular fluid extracts and of corpus luteum, there could not be the equivalent of five rat units in the dosage cited in this paper. The equivalent of only 3.5 grams of the original tissue produced the results reported in this paper, while according to Gustavson it would require 5 cc. of follicular fluid or 77 grams of corpora lutea to give 5 rat units.

SUMMARY

1. Purified extracts were made of sow ovaries from which the follicular fluid and corpora lutea had been removed. The effects were studied in experiments in which about 175 animals were used.

2. When injected into 21-day-old female albino rats, the extract caused precocious sexual development. The males were not noticeably affected.

3. The diameter of the uteri of the experimental animals was two to three times normal in 7 to 10 days.

4. No continuous estrus smear of either young or older animals could be noted except as this smear naturally occurs during a heat cycle. They did not remain in heat longer than the normal length of time.

5. Five rat units of theelin caused sexual maturity in the same time as the purified extract, but did not affect the uteri of the animals.

6. Pituitary bodies from donors that had received the purified extract until their vaginas were opened, were implanted, one daily, for ten days. At the end of this time the vaginas of the receivers were opened. Implants made from animals of same age as above, but receiving pure olive oil, had no effect on their receiver.

7. Histological studies showed more imbedded ova in the ovaries of the experimentals than in the controls, but not conclusively so. As far as could be observed, there was no effect upon the testes of the young males.

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THE RELATION OF CONTRACTURE AND TETANY TO EXPERIMENTALLY PRODUCED CALCIUM DEFICIENCY IN CATS, WITH AND WITHOUT LESIONS OF THE CORTICAL MOTOR AREAS*

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We noted in an earlier series of experiments (2) that animals which had received sodium bromide daily before or after thyroparathyroidectomy, or during both periods, did not show the same fall of ionized calcium or the same decrease in the calcium phosphorus ratio in the same period of time as untreated animals. Furthermore, the period of survival was greater in the animals which received bromides, with no appearance of tetany. There seemed two possibilities of the mechanism of this protective action of bromides, namely,

(1) That the bromide ion has some specific effect upon the calcium and phosphorus metabolism apart from its action upon the central nervous system, or,

(2) That the protective action of the bromide was due to its general cutting down of the motor or other activity of the central nervous system.

It seems possible to test these two possibilities experimentally. If one should make some experimental lesion of the central nervous system which would reduce greatly the general motor activity of the animal and find that such animals live longer, show a less rapid change of ionized calcium and inorganic phosphorus, and are more free from tetany than control cats after thyroparathyroidectomy, it would seem to be presumptive evidence for the second possibility. If, on the other hand, one should find that such experimental lesions of the central nervous system had no measurable or observable effect upon the general deportment of the animals after thyroparathyroidectomy, the results would seem to constitute presumptive evidence in favor of a specific effect of the bromide ion upon calcium and phosphorus metabolism. The purpose of this paper is to present the results of a series of observations upon animals which had undergone simultaneous removal of the thyroparathyroids and some lesion of the central nervous system designed to reduce general motor activity for a period of a few days. The period of a few days is important, since anyone who has had experience with animals with lesions of the central nervous system must be familiar with the rather rapid resumption of motor activity after a lesion of any single motor mechanism.

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In addition to the observations on the deportment of the animals, and the general changes in the calcium and phosphorus, we availed ourselves of the opportunity to study the general working power of the rectus abdominis muscle in these animals, as well as in others. The studies of the deportment of muscle are based upon the results in the following different series of animals:

(a) 15 controls; (b) 10 controls fed on bromide; (c) 15 cats which had undergone simple thyroparathyroidectomy without bromide therapy; tetany and contracture were studied in their relationship to the calcium and phosphorus of the blood serum; (d) 10 cats which had been fed 1 gram of bromide daily for two weeks before thyroparathyroidectomy; tetany and contracture were studied in their relationship to blood calcium and phosphorus; (e) 10 cats which had undergone simultaneous removal of the cortical motor area and thyroparathyroidectomy; tetany and contracture were studied as related to calcium and phosphorus; (f) 5 cats which had undergone double adrenalectomy; contracture was studied as related to calcium and phosphorus.

EXPERIMENTAL RESULTS

(a) *The general deportment of animals with simultaneous lesions of the central nervous system and thyroparathyroidectomy.* The lesion of the central nervous system which would give rise to the greatest loss of motor activity, as opposed to merely uncontrolled and incoordinated motor activity, and at the same time afford the maximum chance of survival of the animal, seemed to be bilateral ablation of the cortical motor areas. This operation does not result in any greater or more permanent paralysis than median longitudinal incision of the midbrain through the decussation of the rubrospinal tracts (6), but the chances of survival are somewhat greater. In this series of operations we paid no attention to whether the lesion was limited strictly to the motor area or to whether the corpus striatum of either side was involved, as our object was to reduce general motor activity of the animal.

The deportment of the animals in the first 48 hours was in all respects identical with the deportment of animals after cortical motor lesions.* It is not to be expected that thyroparathyroidectomy per se will add to or detract from the general picture after cortical motor area ablation. But the persistence of this deportment unchanged for 48 hours after concomitant thyroparathyroidectomy is in contrast to the great lassitude manifested at the end of similar intervals after thyroparathyroidectomy alone. Nor did such extreme lassitude appear in general until after the animals had begun to recover somewhat from the effects of motor area ablation and to move around more freely. As we have observed previously, cats with motor areas intact which were extremely lethargic following the daily administration of bromides for two weeks, would become more active for a few days immediately following thyroparathyroidectomy and the cessation of bromide therapy, or with its continuance.

Tetany. Tetany did not appear in the first few days following the operations. About the sixth day afterward when the animal began to move about more freely tetany might appear. The twitching of isolated groups of muscles about the neck and shoulders was finally apparent in one animal on the sixth day. A series of convulsions appeared on the seventh day, at which time the animal was etherized and the working power of the rectus abdominis muscle tested.

The observations show what might be expected from the second possibility stated above, namely, that the appearance of tetany is measurably delayed after cortical motor ablation, and that it appears within a day or two after the animal begins to move about more freely as it recovers from the effects of the cortical lesion.

The greatest duration of life in animals of this series was seven days. This is significantly longer than after simple thyroparathyroidectomy with no other experimental procedure. The only animal in this or the preceding series which showed evidence of accessory parathyroids was alive six months after bilateral motor area ablation and removal of all that was visible of the thyroids and parathyroids. The mere surgery, in itself, does not seem fatal. The cat died of a general respiratory infection, with the frontal sinuses filled with pus.

Calcium and phosphorus. Calcium and phosphorus were determined at the time the working power of the rectus abdominis muscle was measured. The numerical results are given in Table I, which shows also the

TABLE I

CATS WITH SIMULTANEOUS REMOVAL OF THE CORTICAL MOTOR AREAS AND THYROPARATHYROIDECTOMY

Days After Operation	Ca	P	Ca/P Ratio	Tetany	Contracture	Other Remarks
1	6.4	5.1	1.2	None	Some	Lungs inflamed.
1	9.5	4.1	2.3	None	Control amount	
2	7.2	...	...	None	Some	
2	7.6	6.1	1.2	None	Control amount	
3	7.5	...	...	None	Slight	Lungs inflamed.
3	7.3	6.4	1.1	None	Slight	
4	6.8	...	...	None	Control amount	
5	6.2	...	...	None	Some	
6	5.9	7.9	.7	None	Large	
7	4.8	10.0	.5	Convulsions with spasmophilia	Only control amount.	

presence or absence of tetany and the degree of contracture exhibited by the rectus abdominis muscle. Blood calcium and phosphorus in this series were determined only at the time of operation, not for the pre-operative period, since we have a large number of blood calcium and phosphorus determinations on controls, which have established a mean for calcium of 10.0 mg. for 100 cc. serum with an average deviation of ± 0.5 mg., and, for phosphorus, of 5.5 mg./100 cc. serum with an average deviation of ± 0.5 mg.

The calcium curve, as plotted against the time, shows a more gradual decline than is the case in controls after thyroparathyroidectomy. During

the first 48 hours, when the animals are fairly quiet, the fall of calcium and the attendant rise in phosphorus is only about half what it is in simple thyroparathyroidectomy (calcium usually falls about 50 per cent in the first 48 hours), but by the time motor activity is more completely regained, the calcium has reached the 50 per cent level with the attendant rise in phosphorus. In this respect, the effect of ablation of the motor areas on the blood calcium seems to be more transient than the effect of bromide treatment, since it has been shown that after thyroparathyroidectomy in animals previously treated with bromides, the calcium level remains consistently higher, and the phosphorus level remains lower, than in untreated cats (Table II).

TABLE II

CONCENTRATION OF CALCIUM AND PHOSPHORUS IN SERUM OF CATS, AFTER VARIOUS PROCEDURES

Controls		Bromide Controls		Thyroparathyroidectomy		Bromides and Thyroparathyroidectomy		Adrenals	
Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
9.4	4.9	10.7	5.0	4.6	6.0	7.4	4.8	11.0	5.2
9.7	6.2	11.2	5.0	5.2	4.6	8.0	4.8	10.4	6.0
10.1	4.2	10.1	5.0	5.2	6.2	8.4	6.5	9.9	5.0
10.1	5.4	10.6	6.5	7.0	5.9	7.9	5.7	10.1	6.5
10.5	5.1	11.0	6.0	6.5	6.9	8.2	5.2	10.0	4.8
10.0	5.0	10.4	4.5	5.6	6.0	7.5	2.5		
10.1	6.5	10.0	4.0	4.0	5.7	8.0	6.5		
10.0	4.5	10.0	5.5	6.0	5.0	8.6	4.5		
9.9	4.7	11.0	4.5	5.1	6.2	7.6	4.2		
9.0	4.6	10.2	5.5	4.5	5.1	6.6	4.5		
10.0	5.5			*5.7	5.8				
10.5	6.2			*8.0	5.9				
10.4	6.0			*6.7	6.4				
9.4	5.8			*8.5	5.5				
10.1	5.4			*7.1	6.2				
Average... 10.0	5.3	10.5	5.1	6.0	5.8	7.8	4.9	10.3	5.5
Average Deviation.. ± 0.3	± 0.6	± 0.4	± 0.6	± 1.05	± 0.5	± 0.4	± 0.8	± 0.3	± 0.6

*Parathyroids only.

The deportment of the rectus abdominis muscle in the various series of animals. The various groups of animals on which the deportment of the rectus abdominis muscle was studied have been listed previously. The specific results in each group are given below.

The method of preparation of the rectus abdominis was the same for all groups. Urethane was administered by stomach tube. About an hour later curare was injected intravenously and artificial respiration was maintained by tracheal insufflation. The rectus abdominis muscle was then freed at its upper attachments and dissected down to the lower border of the ribs according to the method described in an earlier paper (5). In the present series of experiments the dissection was so made that most of the blood vessels supplying the muscle were left intact, but the muscle was free to contract throughout its length. As a matter of fact, the contractions of the upper portion of the muscle only were recorded. Fine wires were attached at the upper (cephalic) border and just at the lower

border of the ribs, and shielded so that they did not come in contact with any other muscles. The muscle was kept moist by warmed 0.9 per cent solution of sodium chloride flowing uniformly from a dropper onto its surface. The upper end of the muscle was connected by a thread and pulley to a specially constructed writing lever designed to move vertically upon the kymograph. The muscle was stimulated by moderate induced currents once a second through the two wires attached to it. Blood samples were taken just before the beginning of the stimulation of the muscle.

(1) *Control animals with no other experimental procedure or therapy.* The muscle curve in these animals was uniformly the curve typical for

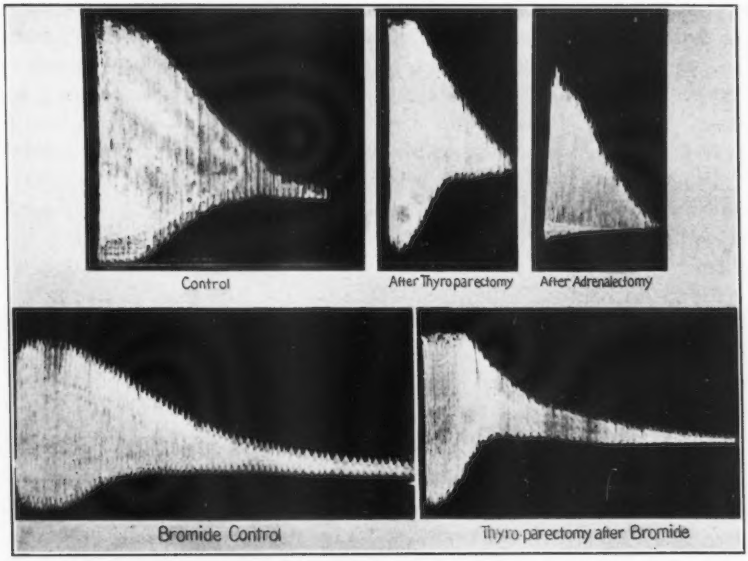

Figure 1. Showing the working power of the rectus abdominis muscle, the appearance and character of contracture, and the failure of response to stimulation. The length of the record is proportional to the duration of the muscular response.

Beginning at the upper left hand, the order is, control animal, animal after simple thyroparathyroidectomy, after bilateral adrenalectomy, bromide control animal, and animal after theyroparathyroidectomy, with bromide therapy.

ordinary striated muscle when stimulated until it no longer contracts. The treppe, contracture and fatigue were present but were not exaggerated (Fig. 1). Intravenous injection of 10 cc. of a 5 per cent solution of calcium gluconate did not produce any noticeable change in the muscle curve of the unfatigued rectus abdominis of the opposite side when the second muscle was stimulated following failure of the contractions of the first muscle. The concentration of ionized calcium for this group showed an average of 10.0 mg./100 cc. blood serum, while the inorganic phosphorus was 5.3 mg./100 cc. blood serum. The ratio of calcium to phosphorus was 1.9.

(2) *Control animals previously fed on bromides but with no other experimental procedures.* The muscle curve of the rectus abdominis was similar to that for the untreated controls. There was no change in the amount or type of treppe, contracture or fatigue. The average concentration of ionized calcium was 10.5 mg./100 cc. serum, and that of inorganic phosphorus was 5.1 mg./100 cc. serum. The ratio of calcium to phosphorus was 2.0.

(3) *Animals after thyroparathyroidectomy, or parathyroidectomy, with no other experimental procedure or therapy.* Tetany, as previously stated, was well-marked within 48 hours after operation. So far as the limits of this experiment are concerned, there were no essential differences between the type of muscle curve obtained after thyroparathyroidectomy and that seen after parathyroidectomy. All of these animals showed a much more exaggerated contracture than the controls. In general, the degree of contracture observed seemed to be related to the calcium deficiency. The earliest onset and most marked development of contracture occurred in those animals in which the fall of calcium had been greatest.

When 10 cc. of a 5 per cent solution of calcium gluconate was injected following the failure of contraction of the first muscle, the contracture in the rectus abdominis of the other side was always much less marked than for the first muscle. The stimulation of the second rectus abdominis was not begun until about thirty minutes after the injection of the calcium gluconate. The average concentration of ionized calcium at the beginning of the stimulation of the first rectus abdominis was 6.0 mg. and that for phosphorus 5.8 mg./100 cc. serum. The Ca/P ratio was approximately 1.0. Toward the close of the experiment, after injection of the calcium gluconate, the average concentration of calcium had increased to 12.5 mg., while the phosphorus remained unchanged. The ratio of Ca/P was then 2.2.

(4) *Thyroparathyroidectomy with previous administration of bromides.* As noted in our previous paper, tetany was of rare occurrence in this series of animals but the rectus abdominis did, however, show the exaggerated contracture seen after thyroparathyroidectomy. The average concentration of ionized calcium in these animals was 7.6 mg./100 cc. serum, and of phosphorus 4.9 mg. The ratio of Ca/P was 1.6. Since the muscles were tested at widely varying intervals after thyroparathyroidectomy, the variations in the concentration of calcium and phosphorus were rather wide. The details of these variations were published in our previous paper. In general, the degree of contracture was inversely proportional to the actual amount of calcium present.

(5) *Thyroparathyroidectomy with bilateral ablation of the cortical motor areas.* The appearance of contracture in this group was variable and was generally slight at most during the first five days. Up to this interval the concentration of calcium did not fall below 6.2 mg./100 cc. serum for any animal. In one cat, the muscle of which was tested out on the sixth day, contracture was observed at a concentration of calcium and

phosphorus of 5.9 mg. and 7.9 mg., respectively. In an animal tested on the seventh day, in which the calcium and phosphorus were 4.8 and 10.0 mg., respectively, convulsions were present before the administration of urethane, but contracture was not greater than in control animals. The striking point concerning this animal is the abnormally low ratio of calcium to phosphorus. The calcium was lower than in many other cases in which contracture was strikingly developed, but the concentration of phosphorus was unprecedentedly high.

(6) *Animals after bilateral adrenalectomy.* Zwemer and Sullivan (7a) have shown that the calcium and phosphorus remain relatively unchanged after adrenalectomy, while the sodium falls very low. In seven animals, two of which were from Dr. Zwemer's series and five from ours, no contracture was observed after double adrenalectomy, but the working period was much shorter than in controls. The calcium to phosphorus ratio, however, was 1.9 or 2—that is the same as in controls.

GENERAL COMMENT

The suggestion that the rate of fall in the concentration of the ionized calcium and inorganic phosphorus of the blood after thyroparathyroidectomy is related to the general activity of the central nervous system is apparently confirmed by the experimental results. Although the effects of ablation of the cortical motor areas is merely transitory, so far as the reduction of motor activity is concerned, it is clearly apparent that in no case did tetany appear until significantly later than in control animals after thyroparathyroidectomy. While bromide therapy may be continued as long as desired, the effects of a lesion of a single motor mechanism cannot be prolonged except by a lesion of another motor mechanism. The effects of a lesion of a second motor mechanism are so severe, even when an interval of some months elapses between the two operations, that cats usually succumb in a few days. Successive lesions of different motor mechanisms were impracticable after thyroparathyroidectomy. The effect of a lesion of an afferent mechanism, such as bilateral vestibular extirpation, which also greatly reduces controlled motor activity for some days, was not tried.

While there might be some reason, as yet unknown, why bromide therapy might decrease the loss of calcium after parathyroidectomy through its action, possibly chemical, upon the organs or tissues through which calcium excretion occurs, and thus slow down the changes in the concentration of ionized calcium and inorganic phosphorus in the blood, it would seem strange that ablation of the cortical motor areas would act upon this same excretory mechanism. The common element in the two general pictures is more the general reduction in the activity of the central nervous system, and through this, of the metabolic changes in the skeletal muscles.

The rate of metabolism of the skeletal musculature seems to bear some relation to the rapidity of the decline in the concentration of ionized

calcium in the blood, although the nature of this relationship is not clear at present.

Whether tetany is the result of metabolic changes occurring in the muscle or whether it is a late event in a series of processes directly concerned with the decrease in the concentration of calcium is a question connected with the problem of the general metabolism after thyroparathyroidectomy. Differently stated, is the tetany merely the result of a decrease in calcium through processes in which it has no causal rôle, or is tetany an event coming at a definite time in the series of changes in the muscle which, in themselves, lead to the reduction of the calcium in the blood? One gets the impression from reading the literature that tetany results when the calcium has fallen to a sufficiently low level as a consequence of general disturbances of metabolism, but that the tetany itself, or other processes in the muscle preceding the onset of tetany, do not bear any necessary relation to the processes responsible for the decline of calcium. The parathyroid hormone is simply stated to have a more or less direct relationship to calcium metabolism, with no statement as to the organs or systems upon which this effect might be manifested. The skeleton is probably the largest repository of calcium in the body, but the remaining calcium, which includes the ionized calcium, must be sought elsewhere. The blood itself and the muscles and possibly the liver must constitute the repository for the remaining calcium in the body. Other tissues, because of the smallness of their bulk, could account for only a small percentage of it. The study of the changes of calcium shows that it increases rapidly in the blood following convulsions of experimental origin. It seems questionable whether this mobilization could occur so quickly from the bones. There is a possibility, perhaps unproved as yet, that the skeletal, and possibly other, muscles liberate calcium during activity. If they do, what becomes of the calcium?

If the muscles are the source in part, or wholly, of the calcium that is excreted, a higher general level of muscular activity might be expected to lead to the more rapid decline of calcium and the earlier onset of tetany than a lower level of muscular activity. Tetany would, then, be an accompaniment of a certain stage in the history of the muscular changes following parathyroid removal, and this stage would be the result of previous processes occurring in the muscle itself.

Tetany does not appear in muscles in which the nerve supply has been interrupted anatomically. Two known conditions, in addition to others still unknown, are necessary for its appearance—a change in the muscle itself, and the conservation of its efferent nerve supply.

The necessity for the presence of two known conditions in the onset of tetany comes out in the study of the working power of the muscles. The results on the working power of the muscles show, in their turn, another phase in the history of the changes in the muscles. It is generally agreed by all observers that, in muscles from normal or control animals, some

degree of contracture appears as an incident on the road to complete failure of the muscle to respond to stimulation. Ordinarily, the first appearance of contracture does not occur until there is a significant or at least, perceptible, drop in the height of the contractions. It develops slowly until it reaches its maximum development, after which the point to which the muscle relaxes remains relatively constant, while the height of the contraction slowly decreases. This statement is more particularly true of the rectus abdominis muscle *in situ* than of excised frog muscle. After thyroparathyroidectomy the total working time of the muscle is greatly reduced and the interval elapsing between the beginning of the working period and the appearance of contracture is significantly shorter. The slope of the curve indicating the genesis of contracture is considerably steeper and the degree of relaxation of the muscle much less than in controls. Furthermore, instead of remaining horizontal after reaching the maximum, as in controls, the line indicating the greatest relaxation of the muscle may rise almost continuously until the practical failure of the muscle to contract at all. This picture is well developed 48 hours after thyroparathyroidectomy in controls without other experimental measures. Tetany is always present at such times. The same degree of contracture was observed in one animal 48 hours after simultaneous excision of the thyroids and bilateral ablation of the cerebral motor cortex, but no tetany was ever seen in this animal. The concentration of calcium was 6.4 and of phosphorus, 5.1 mg./100 cc. serum. The Ca/P ratio was 1.2. Intravenous injection of calcium gluconate, while it did not significantly affect the total working time of the muscle, greatly reduced the degree of contracture in the opposite rectus abdominis. In other animals of this series, contracture 72 hours after the double operation was scarcely greater than one would expect in a control animal, while in another animal, contracture 7 days after operation was no greater than in a control. This last animal is of particular interest for, although there was no continuous tetany at any time, and no significant increase in contracture, the animal manifested periodic onset of twitches of whole muscle groups, usually appearing in the fore limbs and about the head, which developed into a generalized clonic convulsion. The twitches might appear thirty minutes before the onset of a generalized convulsion which, in turn, would last about five minutes. A period of three or four hours, during which the animal was free from twitches or convulsions, would then follow. The concentration of calcium was 4.8 and of phosphorus was 10.0 mg./100 cc. serum, with a Ca/P ratio of 0.48. The working power of the muscle and its total working period were significantly greater than for any other animal with a concentration of calcium even approximating such a low level. Whether the freedom from tetany with its replacement by periodic spasmodic phenomena, the greater working power, and the greater working time, were related to the abnormally high concentration of phosphorus, or the very low ratio of Ca to P, is problematical.

The appearance of contracture in control animals fed with bromides does not differ significantly from that in control animals without any other procedure. Nor does the contracture, when it appears following thyroparathyroidectomy with bromide therapy, differ significantly from that appearing after thyroparathyroidectomy alone. The mere presence of bromide, in itself, apparently does not affect contracture. But the interval elapsing between thyroparathyroidectomy and the time when contracture in the muscle is well-developed may be 7 to 14 days longer than the similar interval without bromide therapy. The highest concentration at which a greater than control degree of contracture was observed was 7.5 mg. of calcium, and 6.1 mg./100 cc. serum, of phosphorus.

The greatly reduced working power of the muscle is a striking feature following bilateral adrenalectomy. The total working period may be even less than that after thyroparathyroidectomy, but contracture is, even in the most pronounced cases, only moderate in degree and generally of slow onset, while in others it may be completely absent, the muscle relaxing to the base line until complete failure of contraction occurs. Calcium is neither increased nor reduced in these animals, but the phosphorus, however, may show somewhat greater variation. The significant changes are the great reduction in the concentration of sodium and the general fall in the level of blood sugar. The only animal in the series which showed contracture approaching that seen after thyroparathyroidectomy was one in which the concentration of sugar in the blood remained relatively high.

While we have no direct estimation of sugar in the blood of animals the working power of whose muscles was tested after thyroparathyroidectomy, such observations as we have on any animals of the thyroparathyroidectomy series show a relatively high concentration of sugar. While the data are suggestive, we do not consider them sufficient as yet to show any necessary relationship between the concentration of sugar and the appearance or non-appearance of contracture. In general, the variables entering into the general activity of muscle (1, 4) seem far too many to permit of any very definite statements of a causal relationship on the basis of the estimation of only a few of them at one time.

SUMMARY

1. An injury of the central nervous system, such as bilateral ablation of the cortical motor region, which reduces general motor activity after thyroparathyroidectomy (a) prolongs the life of the animal; (b) defers significantly the onset of tetany; (c) slows the rate of fall in the concentration of ionized calcium in the blood; (d) has a less uniform effect on the concentration of inorganic phosphorus; (e) defers the general change in the physiological condition of the rectus abdominis muscles, as measured by its working power, the total time during which contraction is maintained, and the appearance of contracture.

2. The results suggest, although they do not completely prove as yet, that the general metabolism of the striated musculature is a factor con-

cerned in the genesis of the train of events following parathyroidectomy. It seems possible that the time of appearance of the various symptoms varies inversely as the speed of metabolic processes in the muscle.

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DIURESIS ASSOCIATED WITH DIRECT STIMULATION OF THE HYPOPHYSIS

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Experimental lesions in the hypothalamus in cats have been found in this laboratory (Fisher, Ingram and Ranson, 1934) to be associated with permanent polyuria only when the lesions are placed so as to interrupt the tractus supraoptico-hypophyseus, which courses down the infundibular stalk to the pars posterior and pars intermedia of the hypophysis. Animals with permanent polyuria show marked atrophy of the supraoptic nucleus and of the posterior lobe. The polyuria resulting from such lesions occurs in two phases: a transient phase lasting one to four days after operation, and a permanent phase which comes on after a latent period of 8 to 15 days. The permanent polyuria seems to be a deficiency phenomenon, but it is not unlikely that the transient diuresis may be due to irritation, possibly of one of the lobes of the hypophysis. It will be recalled that Weed, Cushing and Jacobson (1913) observed diuresis and glycosuria in catheterized rabbits and cats following stimulation of the hypophysis and after stimulation of the superior cervical ganglion. A definite diuresis resulting from stimulation of the superior cervical ganglion was established by Shamoff (1916). The results of Cushing and his co-workers have recently been confirmed by Davis, Cleveland and Ingram. Keeton and Becht (1916) stimulated the hypophysis electrically in dogs and found elevation of the blood sugar, but only intimated a diuresis which was presumably due to the glycosuria. Hanchett (1922) observed transient polyurias in dogs following production of irritative lesions in the hypophyseal region or stimulation of the hypophysis with faradic current. Schäfer (1911) also found mild transient polyuria after irritative hypophyseal lesions. None of these investigators was specific as to which of the hypophyseal lobes was involved, although Cushing assumed that the posterior lobe was the one in question. The present authors have attempted to stimulate the anterior lobe of the hypophysis in cats, taking advantage of the opportunity offered by the use of the Horsley-Clarke stereotaxic instrument, which enables one to introduce a fine bipolar needle electrode into the gland from above. The hypophysis is not directly exposed and the fine needle produces a minimum of damage to overlying brain structures.

METHODS

The experiments were performed in the following manner: A blood sample was taken from the saphenous vein and the cat anesthetized by intravenous injection of pentobarbital sodium (28 mgm. per kilo). The

bladder was catheterized and the catheter arranged so that the urine dripped into a tube graduated in tenths of a cubic centimeter and containing a crystal of thymol. A quantity of normal salt solution (50 to 100 cc.) was injected intraperitoneally in some cases, in others 50 to 100 cc. of water were given by stomach tube. Ordinarily a period of 30 to 90 minutes was allowed to elapse between the time of anesthetization and operation, during which time the bladder was drained and usually another blood sample taken. The operative procedures consisted of opening the skull and adjusting the stereotaxic instrument to the head. After the bladder had once been emptied and the flow of urine had become uniform, the quantity of urine passed through the catheter in each fifteen-minute interval was noted. Stimulation of the hypophysis was not begun for from one to three hours after operation, during which time one or more blood samples were taken. If during this interval the rate of flow of urine remained fairly constant and within normal limits, a fine bi-polar needle electrode was inserted, from above, through the substance of the brain, into the hypophysis. The stimulus was a faradic current derived from a Harvard inductorium connected with one dry cell, and with a secondary coil separation of 7 to 9 cm. There is very little spread with the fine type of bipolar electrode used, since the two poles are separated by only 0.2 to 0.4 mm. The stimuli were ordinarily applied for two to six minutes. There were usually several periods of stimulation in the course of an experiment and sometimes the electrode was shifted to different spots in the hypophyseal region for successive stimuli. During the later experiments procedure was to begin stimulation while the tip of the electrode was placed well dorsad in the hypothalamus; then successive stimuli were applied as the needle was lowered millimeter by millimeter into the hypophysis. By this method one could withdraw the needle after positive results had been obtained, insuring subsequent location of the point stimulated since the latter would, of course, coincide with the end of the puncture produced in the hypophysis or tuber. Hereafter we shall use the expression dorsal approach to indicate the use of the stereotaxic instrument and ventral approach for the following type of experiment.

In a number of experiments the hypophysis was exposed through a drill hole in the spenoid bone and the gland stimulated by plunging a needle electrode into its ventral surface. The electrode was manipulated by a holder with a rack and pinion arrangement which enabled one to determine the depth to which the tip of the needle entered the gland. In these experiments the sequence of events was as outlined above.

The cats used were not fed for 24 to 48 hours preceding the experiments.

Blood samples were taken from time to time between stimulation periods.

The Randle-Grigg modification of the Folin-Wu technique was used for the blood sugar determination. Qualitative tests for the presence of

reducing substance in the urine were made according to the method of Benedict.

At the end of an experiment the cat was killed by bleeding and the brain fixed and hardened by injecting the head with formaldehyde (U.S.P. 1:10). The brain, with the hypophysis attached, was removed and the hypothalamic-hypophyseal region embedded in celloidin or paraffin, sectioned, and every fourth section stained alternately with cresyl violet and Weil's method.

RESULTS

Experiments were performed upon 27 cats. Five of these, including preliminary experiments and those in which technical difficulties were encountered, were abandoned. The results of the 22 experiments to be reported may be epitomized as follows:

In 11 cats definite diuresis was associated with stimulation of the hypophysis. In four of these, Cats C, E, J, and N, the diuresis was not associated with glycosuria or hyperglycemia. In Cats C, E, and J the hypophysis was stimulated from the dorsal approach, in Cat N it was exposed and stimulated directly. In each instance when the stimulus was properly applied, the output of urine per fifteen-minute interval was markedly increased, the volume output increasing as much as 100 to 740 per cent. The normal urine flow averaged about 0.5 cc. per 15 minutes; following stimulation as much as 4.0 cc. were passed. There was no glycosuria in any case. Histological examination revealed that during the effective stimulation the tip of the electrode was placed near the point of juncture of pars tuberalis and pars anterior, so that the stimulus could have affected pars tuberalis and the dorsal surface of the anterior lobe. Stimulation in the body of the anterior lobe, in the hypothalamus or posterior lobe had no effect on diuresis. Figure 1 presents the results for Cat N. Two of these cats, E and J, showed slight increases in blood sugar following stimulation.

In the other seven cats of this group, D, F, H3, L, M, H, and I, the diuresis was accompanied by glycosuria. The hypophysis was stimulated from the dorsal approach in all of them. Marked increases in urine output were produced in each cat. In five cats, following decline of the urine flow to normal, the diuresis was re-elicited by repeated stimulation; in two of these the repeated stimulation was effective on three occasions (Fig. 2). In only one cat (Cat F) was stimulation associated with elevation of the blood sugar, which rose from 130 to 319 mg. per cent; in the others glycosuria was present before stimulation. Examination of the histological material showed that the site of stimulation effective in producing diuresis was in the region of the pars tuberalis and the dorsal surface of the anterior lobe, stimulation elsewhere in the gland having no diuretic effect. In two cats, however, while the tip of the needle was in the pars tuberalis, the pars anterior was not touched. The anterior lobe was definitely af-

fected in Cat F, which showed elevation of blood sugar, but in this case there was a possibility of spread to the hypothalamus.

In five cats there was glycosuria without diuresis. In four of these, Cats K, H7, H8, H4, the hypophysis was stimulated from the dorsal approach; in Cat Q the gland was directly exposed. No clear-cut diuresis was produced in any instance. In cats K, Q, H8 and H4 glycosuria was present before stimulation, but in one of these, Cat H4, there were eleva-

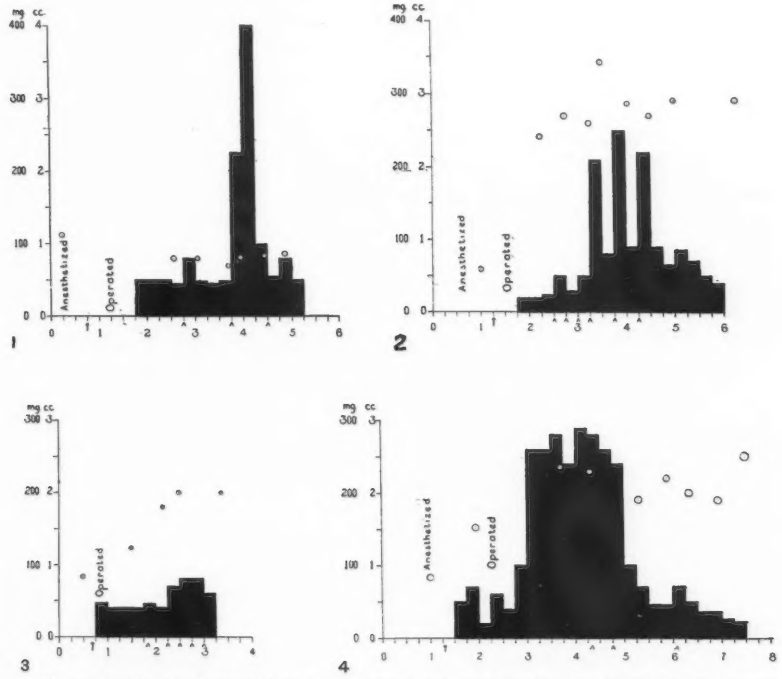

Figure 1. Graph showing blood sugar levels and urine output per fifteen-minue interval in Cat N, in which diuresis was produced without accompanying glycosuria.

→ indicates time of administration of fluid.

∧ indicates time of stimulation.

Figure 2. Graph showing blood sugar levels and urine output per fifteen-minute interval in Cat H3, in which diuresis was produced and in which there was glycosuria.

Figure 3. Graph showing blood sugar levels and urine output per fifteen-minute interval in Cat H7, in which there was glycosuria without diuresis.

Figure 4. Graph showing blood sugar levels and urine output per fifteen-minute interval in Cat P, in which there was spontaneous diuresis and glycosuria.

tions of the blood sugar level which were associated with stimulation of the anterior lobe. In the other cat, H7, the blood sugar was raised from 120 to 200 mg. per cent following stimulation (Fig. 3). The stimuli may possibly have reached the neighborhood of the pars tuberalis in three of these animals, as shown by histological preparations; in two the pars tuberalis was not approached by the electrode. The pars anterior was stimulated in each instance.

In one cat no diuresis or glycosuria was observed.

In five cats spontaneous diuresis and glycosuria were noted. The hypophysis was exposed by the ventral route in Cats O, P, and T; in Cats H1 and H2 the dorsal approach was used. In each of these marked spontaneous diuresis and glycosuria appeared directly after operation and persisted from one to three hours (Fig. 4). Once the urine output had fallen to normal, stimulation of the hypophysis failed to re-induce diuresis. This may perhaps be accounted for in part by the fact that in none of these animals did the electrode approach the spot from which diuresis had been obtained in other experiments. Although the blood sugar levels were high throughout the experiments, in two cats, H1 and T, there were indications of slight rises associated with stimulation of the anterior lobe.

Five control cats were anesthetized, catheterized and observed in a manner similar to the experimental animals. No diuresis was observed (Fig. 5). In four animals there was glycosuria following anesthetization. In one there was no glycosuria and the blood sugar was found to be below threshold level.

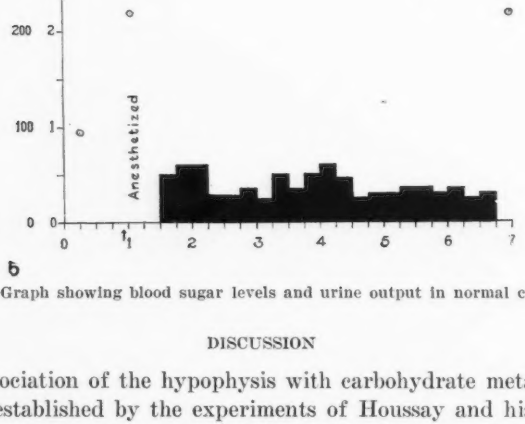

Figure 5. Graph showing blood sugar levels and urine output in normal control 1.

DISCUSSION

The association of the hypophysis with carbohydrate metabolism now seems well established by the experiments of Houssay and his co-workers (1931-1934), Lucke and co-workers (1933-1934), Regan and Barnes (1933), D'Amour and Keller (1933), Evans (1933), Boehm (1932), Mahoney (1934) and many others. The rôle played by the hypothalamus is not definitely established, although Himwich and Keller (1930), Magoun, Barris and Ranson (1932) and Miki (1932) have found the blood sugar level to be elevated following stimulation of the hypothalamus in anesthetized animals, and D'Amour and Keller (1933) observed hypoglycemia after bilateral lesions had been placed in the chiasmal region. Macleod and Donhoffer (1933) are dubious as to the relation of the hypothalamus to carbohydrate metabolism, although they have observed hyperglycemia in

rabbits after decerebration in the region of the pons. Unfortunately our results are not such as to enable us to contribute much of significance to the problem in general. Due, apparently, to the conditions of the experiment, the unsuitability of nembutal anesthesia, and possible slight asphyxia during operation, the blood sugars were usually high and glycosuria present before any stimuli were applied to the hypophysis. In most cases no definite association could be made between stimulation and glycosuria. A total of 17 cats showed glycosuria, in 15 of which the glycosuria appeared before stimulation of the hypophysis; in two only the glycosuria appeared after stimulation. Elevations of blood sugar which in the animals of this group seem to be associated with stimulation were found in five cats, F, H1, H4, H7, and T. Of the four cats which showed diuresis without glycosuria upon stimulation, two showed increased blood sugar following stimulation, Cats E and J, but in these the figures remained below threshold level. In Cat E the tip of the needle electrode was in the immediate neighborhood of the pars tuberalis and the surface of the anterior lobe. In Cat J the stimulus which was effective in regard to the blood sugar must have been applied near the periphery of the gland and perhaps affected the hypothalamus. In Cat F stimulation of the anterior lobe only was associated with hyperglycemia. In Cat H7 the effective stimuli affected the hypothalamus and anterior lobe. In Cats H1, H4, and T the anterior lobe only was stimulated.

The workers who have reported diuresis or polyuria following stimulation or irritation of the hypophysis have already been mentioned. All of these with the exception of Schäfer and Hanchett have noted that the diuresis was accompanied by glycosuria. That this need not always be the case is shown by Cats E, C, N and J of the present series, in which definite diuresis not associated with glycosuria was observed. It is of interest that the sites of stimulation in these instances have been found to lie in the dorsal portion of the gland, usually with the electrode impinging upon the pars tuberalis. Stimuli deeper in the gland were without effect in producing diuresis. Stimuli outside the hypophysis were also without effect.

In seven cats diuresis was observed in the presence of glycosuria. In six of these the glycosuria was present prior to the stimulation, and further evidence that the two are not necessarily associated is found in the fact that the glycosuria may persist after the diuresis has subsided. In these cats again the superficial location of the point of effective stimulation should be noted. In Cat F, diuresis occurred only when the needle was in the neighborhood of the pars tuberalis. In Cats D and I, the pars anterior was not touched by the electrode while the pars tuberalis undoubtedly was. In Cat H, the effective puncture penetrated the pars tuberalis. In Cats L, M, and H3, the needle was placed close to the pars tuberalis. That the occurrence of diuresis in these animals was no mere coincidence is indicated by the fact that in several cases the response was reinduced by

repeated stimulation. This was true in Cats D, F, H3, M, and H. In those in which the responses were most marked the rate of flow dropped to normal levels between the successive effective stimuli.

The occurrence of several cases of spontaneous diuresis would appear to cast some doubt upon the validity of the foregoing results. The cause of this spontaneous diuresis is by no means clear. In each instance it occurred rather early in the experiment, apparently coinciding with or immediately following the time of operation and was always associated with glycosuria. It should be noted that Keeton and Becht observed glycosuria as a result of exposure of the gland, and in three of our five cases the buccal approach to the hypophysis was used. In the other two, however, the diuresis followed adjustment of the stereotaxic instrument, in which the gland was not approached. It is conceivable that pressure upon the neck in such a way as to stimulate the superior cervical ganglion might produce these phenomena, but manual massage of the cervical region in unoperated controls was without effect. Certain features of this diuresis perhaps serve to differentiate it from that occurring after stimulation of the hypophysis. These are its early onset and relatively prolonged existence. In all the experiments sufficient time was allowed after operation for the occurrence of spontaneous diuresis, and stimulation was not ordinarily begun until a fairly uniform flow of urine within normal limits was established. Furthermore, the diuresis produced by stimulation was of short duration, the urine output dropping quickly to normal. That repeated stimuli could reproduce the diuresis in certain cases also speaks against the possibility of coincidence.

It will be recalled that frequently the diuresis could not be called forth a second time, or that successive increases in urine output were often less intense than that produced initially. This is in line with Shamoff's experience in stimulating the superior cervical ganglion. It was also found difficult to produce diuresis by stimulation after a period of spontaneous diuresis; in these cases, however, the stimulated areas did not correspond with those in positive cases, the electrode usually not approaching the dorsal portion of the hypophysis.

The six experiments in which no diuresis followed stimulation, although glycosuria was present, are not readily explained, except perhaps by assuming that in any series of experiments there may be a number of failures. The matter becomes more puzzling in view of the fact that in three of these cases there was a possibility of the stimulus reaching the region found to be crucial in positive experiments. In two, this area was not approached. The sixth case is interesting because of the absence of glycosuria and the presence of a destructive occlusion at the mouth of the infundibulum.

The rapid onset and short duration of the periods of diuresis are rather surprising if one assumes the effects to be due to release of hormonal substances. However, stimulation of nervous structures in the adjacent

hypothalamus has not been found to produce diuresis and the closely adjacent hypothalamico-hypophyseal fiber system is more likely to be related to the prevention of excessive water loss, since it has been found to be degenerated in animals with polyuria. Other nervous elements which might be brought into question are the fibers which are said to course down blood vessels surrounding the infundibular stalk to innervate the anterior lobe (Dandy, 1913). The possibility that stimulation of these fibers might have effects which could not be produced by direct stimulation of anterior lobe cells cannot be excluded. On the other hand, it must be pointed out that the effective stimuli were also placed in very close proximity to the pars tuberalis, functions of which have not as yet been elucidated.

Whether the diuresis might be associated with elevations of blood pressure has not been determined, although Shamoff found a slight depressor effect followed by a rise in blood pressure after stimulation of the superior cervical ganglion. The effects of operative interventions elsewhere in the nervous system have not as yet been studied, but it should be recalled that Keeton and Becht found it impossible to produce glycosuria after section of the splanchnic nerves. Weed, Cushing and Jacobson, and Shamoff, on the other hand, found that vago-sympathetic section and section of the spinal cord at the fourth thoracic segment did not interfere with their results.

So far as the present results go, however, the authors believe that they are sufficiently convincing to enable one to suggest that transient polyurias may be associated with irritation of the dorsal peripheral region of the hypophysis, in the neighborhood of the pars tuberalis.

SUMMARY

In 11 of 16 cats anesthetized with sodium pentobarbital, diuresis was produced by faradic stimulation of the hypophysis. In four of these the diuresis was not accompanied by glycosuria. In seven glycosuria was present, but in only two did this appear after hypophyseal stimulation, the other cases showing glycosuria before the stimulation was begun. The five cats in which no diuresis appeared also showed glycosuria. In one other cat neither diuresis nor glycosuria appeared.

Five other cats showed spontaneous diuresis and glycosuria. This was distinguished from the diuresis appearing upon stimulation by the fact that it appeared soon after operation and persisted for a considerable period of time.

Histological examination of tuber and hypophysis in all these animals indicated that the point of application of the stimulus producing diuresis was on the superficial aspect of the anterior lobe in close proximity to the pars tuberalis. Stimulation deep in the anterior lobe was not found to be associated with diuresis, but was frequently related to elevations of blood sugar.

It is concluded that the transient polyuria which immediately follows operation for production of experimental diabetes insipidus is most likely due to irritation of the hypophysis around the point mentioned above.

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CASTRATION ATROPHY AND THEELIN

EFFECT OF THEELIN ON ATROPHIC UTERI OF CASTRATED ALBINO RATS

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In the clinical use of theelin, particularly for the relief of the symptoms of menopause, the results reported seem to be sufficiently variable and incomplete to warrant a careful experimental study of the effect of this substance under controlled conditions.

Werner and Collier (1, 2) have reported results following the administration of theelin to a series of ten women in artificial menopause. In addition to demonstrating its value for the alleviation of the symptoms of this condition, they have shown also its effect on the uterine endometrium of their patients. From material secured by curettement, both before and during the periods of injection, they were able to demonstrate a definite hyperplasia and restoration to virtual normal appearance of these surface scrapings of endometrium.

In a previous paper the authors (3) reported the results of a series of experiments in which a chronological study was made of uterine atrophy following removal of both ovaries of albino rats. Comparisons were made, both as to size and histological picture, between normal and atrophic horns. These comparisons included the uterus as a whole and the several coats separately. Using the same procedure, and based upon the findings of that experiment, another series of investigations has been carried out in which we have attempted to show the effect of theelin upon the uteri of such castrates.

The albino rat, having a bicornuate type of uterus, lends itself admirably to this type of study, since one horn could be removed and examined at the beginning of each experimental period and the opposite horn allowed to remain until the expiration of that period. Thus, by having both a control and an experimental uterine horn from the same animal, the variables could be reduced to a minimum.

Young, sexually mature, virgin rats were used in all cases. Both before and after ovariectomy, all animals were examined for cyclic changes by the vaginal smear method. The designation of stages in Long and Evans (4) was followed. All animals were operated in stage 5 or interval of the oestrous cycle. The theelin* used was the aqueous product, and was injected intramuscularly in daily doses of 5 rat units. Vaginal smears taken during the injection periods showed an attempt at oestrous, but no true stage 3 or oestrous stage was produced by the theelin as given.

*The theelin was furnished by Parke, Davis & Company.

Three objectives were sought: to test the effectiveness of theelin in restoring to normal the atrophic uteri of castrates; to test the effectiveness of theelin in preventing uterine atrophy following castration; and to test the duration of the effect of theelin following cessation of the injections.

THEELIN AS A RESTORATIVE

Twenty rats, in groups of four each, were bilaterally ovariectomized, after which their uterine horns were allowed to atrophy for periods of 14, 21, 28, 35 and 42 days, respectively. All four rats in each group had the same period of atrophy. In each group of four rats, one was given 5 daily injections of theelin; a second, 10; a third, 15; and a fourth, 20. At the beginning of the respective injection periods, one horn of each rat was removed as an atrophic control, while the other was allowed to remain in place for the duration of the period, and then was removed and used to show the effect of theelin injections.

Segments were taken from the midregion of each horn and sectioned for study. Microscopic measurements were made, respectively, of the thickness of the endometrium, myometrium, total uterine wall, lumen and total diameter of the horn, both wall and lumen. The stated dimensions represent averages of the four quadrants of a section of each horn, except that the diameters of the lumen and total horn represent averages of the longest and shortest dimensions, respectively.†

These measurements show that, in almost every case, the restored horn was markedly thicker than the atrophic horn, the former falling well within the range of normal uteri, as shown in the previous report cited above. This return to practically normal size also was shown by a series of micrometer caliper measurements, in situ, of the total diameters of the respective horns, taken at the three significant periods of the experiment, viz.: at the time of ovariectomy (normal), at the end of the period of atrophy (atrophic) and at the end of the injection period (restored). These measurements indicate that theelin restored the horns to practically their normal diameters, after having passed through an atrophic stage of definitely reduced diameters. This restorative effect appeared as definite after a 42-day period of atrophy as after 14 days. Five daily injections of 5 rat units of theelin seemed to be sufficient for optimum effect.

Changes in the histologic structure (Fig. 1) of the endometrium were even more marked than were the changes in size of the cornua. In the atrophic (control) horns, the lumen epithelium was greatly decreased in height (to a virtual squamous in some cases); mitotic figures disappeared and there was manifested considerable nuclear degeneration. Similar modifications occurred in glandular epithelium, but to a less extent. There appeared a general descrease in the size of many glands while others became cystic and distended with fluid. Their number also appeared to be fewer than normal. In the endometrial stroma, the cellular elements

†Copies of the detailed tabular data may be obtained on request.

appeared to be crowded toward the lumen and much condensed. There was a marked invasion of the endometrium by fibrous tissue. Many of the cells of the endometrial stroma showed degenerative changes, especially in the nuclei. There was some sclerosis of blood vessel walls.

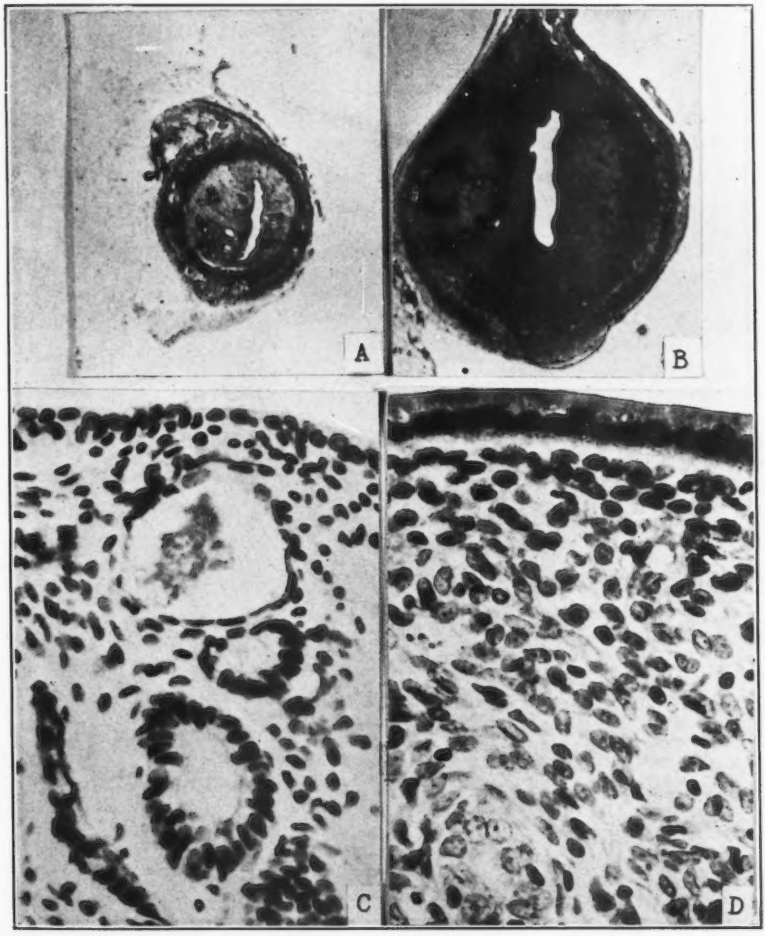

Figure 1. A, low power microphotograph of right uterine horn (rat number 128) taken 42 days after ovariectomy; B, left horn of same rat taken after 5 daily injections of theelin (same projection); C and D, high power microphotographs of A and B, respectively.

The opposite horn, removed after the administration of theelin, showed a greatly altered picture. The lumen epithelium was of normal columnar type; the pycnotic condition of the nuclei had disappeared and many of the cells showed mitotic figures. The uterine glands seemed to be increased in number and normal in appearance; no cysts were observed in

these horns. The crowding of the cells of the endometrial stroma was much lessened, and the cells appeared normal in every particular. However, much of the fibrosis, mentioned above, remained.

Changes in the myometrium, in either case, were few and insignificant.

AS A PREVENTIVE

In this series, 16 rats were subjected to laparotomy and both ovaries and one cornua of the uterus removed, the latter being used as a normal control. On the second post-operative day, injections of 5 rat units of theelin per day were begun and continued for successive periods of days, in multiples of 5 from 5 to 40. The remaining horn was removed at the ends of the respective periods of injection. Two animals were used for each injection period. Sections of both series of cornua were prepared for study; measurements were made and averages computed as in the previous series.† The measurements indicate that, while the normal size of the experimental cornua is not maintained in every case, there was a definite tendency toward such. The average diameter of the experimental horn was 2.07 mm., while that of the normal control horn was 2.23 mm.

Histologically, there appeared little difference between the normal and the theelin-supported horns. In fact, a slight flattening of the lumen epithelium in three rats and a slight crowding of the endometrial stroma cells in an occasional animal were the only deviations from the strictly normal condition. Examination of Figure 2 will illustrate this comparison.

DURATION OF EFFECT

In this phase of the investigation two series of animals were used. In the first, consisting of 10 rats, both uterine horns were left in situ for 40 days following ovariectomy. Following this forty-day period of atrophy, 10 daily injections of 5 rat units of theelin were given. At the end of the injection period one horn was removed and sectioned to show the degree of restoration toward normal. At intervals of 3, 5, 7, 9, 12, 17, 22 and 32 days, respectively, following the withdrawal of theelin, the remaining horn was removed, sectioned and studied in an effort to determine the approximate time at which atrophy would again be manifested. Microscopic measurements were taken for total diameter of the uterus only and were obtained by averaging the longest and shortest diameters of the respective cross-sections of the cornua.† Micrometer caliper measurements are also included.

In the second series of 7 animals, the same general procedure was followed with the exception that no period of atrophy was allowed; the injections of theelin were started on the second day following ovariectomy, and continued for 40 days. At the end of the injection period one horn was removed to show the restored condition of the uterus. At intervals of 29, 31, 36, 42, 52 and 63 days, respectively, following withdrawal of theelin, the remaining horn was removed, sectioned and examined. Microscopic measurements were made as in the previous series.†

†Copies of the detailed tabular data may be obtained on request.

Both statistical and histologic comparisons of these series of cornua indicate that marked variations occurred in the degree of maintenance of the theelin effect. The earliest definite changes were seen about 12 days following the withdrawal of theelin. In many animals, however, the effect of the theelin appeared to be sustained for much longer periods.

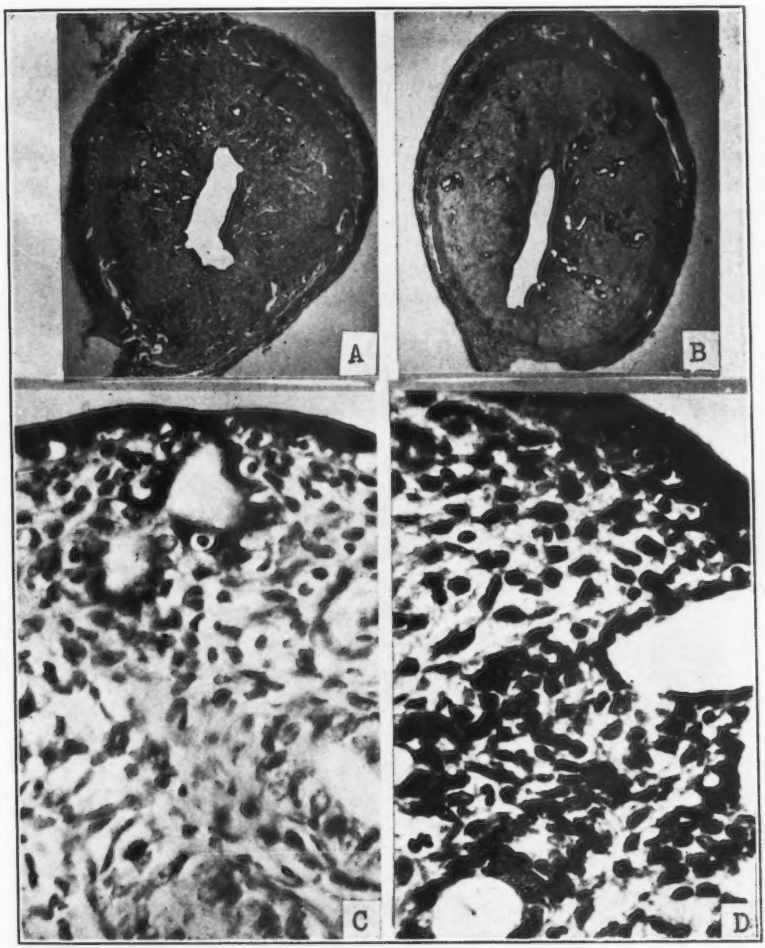

Figure 2. A, low power microphotograph of right (normal) horn (rat number 151) removed at ovariectomy; B, left horn of same rat taken on the 37th post-castration date and following 35 daily injections of theelin; C and D, high power microphotographs of A and B, respectively.

In work previously reported by the authors (3) on castration atrophy in the albino rat, it was found that definite uterine atrophy following castration occurred on about the 14th day after the removal of the ovaries. The results here stated would indicate that the effect of the theelin injec-

tions in the stated doses would have about the same duration as that normally supplied by the ovaries, although it would appear that in some cases the effect of injections would last much longer.

SUMMARY

Fifty-three albino rats were subjected to bilateral ovariectomy and divided into three groups. The first group was used to test the effectiveness of theelin in restoring atrophic uteri to normal; the second, in preventing atrophy following castration; and the third, to test the duration of the effect of theelin.

In all cases opposite uterine horns were used as controls. Measurements were taken of the several coats separately and of the uterus as a whole. Observations were made on changes in histologic detail.

It was found that theelin, in daily intramuscular injections of 5 rat units, produced the following results: (1) It was effective in restoring the castrate uterus after at least a 42-day period of atrophy—5 daily injections being as effective as 20. (2) It prevented atrophy for at least 40 days following castration, showing a tendency to maintain normal size but not doing so constantly. (3) The duration of the effect of theelin was roughly that of the hormone normally supplied by the ovaries, but in some cases was greater.

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A STUDY OF THE RESPONSE OF THE HEART TO PITRESSIN FOLLOWING THE ADMINISTRATION OF THYROID EXTRACT

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In 1928 Kamm and his co-workers (1) succeeded in separating the oxytocic from the vasopressor principle in the extract of the posterior lobe of the pituitary gland. Since then the action of the latter substance upon the arterial blood pressure has been extensively studied (2). Evidence has been adduced to show that it has a constrictor action upon the coronary vessels (3) and that it simultaneously causes certain definite changes in the electrocardiograms taken from experimental animals (4).

It was first demonstrated by Clark (5) and later confirmed by Appel (6) that the sensitivity of the rabbit to pitressin is enormously enhanced by previous administration of thyroid extract. Clark (5) fed rabbits which had tolerated 0.3 cc. of "vasopressin" intravenously without the slightest symptoms, 15 grains of dried thyroid preparation daily for periods varying from seven to twenty days. At the end of about a week or so of thyroid feeding the animals were tested every two or three days with 0.2 cc. of "vasopressin" intravenously. This dose proved to be fatal after a variable period of thyroid feeding. Clark believed the cause of death to be cardiac failure and that vasoconstriction of the coronary vessels was one of the contributing factors.

That the injection of pitressin produces no permanent cardiac damage was demonstrated by Gruber, Oleh and Blades (7). Although some investigators still question whether or not thyroid feeding produces characteristic changes in the heart, many investigators have demonstrated definite changes in the electrocardiograms of experimental animals following total thyroidectomy and following the administration of massive doses of thyroid extract (8).

We have undertaken in this work to show on the electrocardiogram what effect intravenous administration of pitressin produces following the administration of thyroid extract.

METHOD

Non-anesthetized rabbits weighing from 1.7 to 4 kg. were used. The animals were placed in the same lying posture in all of the experiments. The hair on the left fore and hind legs was shaved off to establish better contact with the electrodes. These electrodes were German silver metal plates 6 cm. by 9 cm., curved enough to allow them to fit the contour of

the extremities. When in place they were wrapped with towels moistened with a saturated solution of sodium chloride to establish still better contact. Since we only wished to compare results, Lead III alone was used. A portable Sanborn string galvanometer without amplification was used in recording the action current of the heart. The movements of the string were photographed according to the methods commonly employed. The tension on the string was such that 1 mv. gave an excursion of 1 cm. on the photographic record. A time marker indicating one-fifth of a second was used.

In each case the animal had served as its own control, i.e., before thyroid feeding was started the animal was placed on the board and an electrocardiogram taken of its normal heart and following the intravenous administration of 0.3 cc. of pitressin. The drug was injected into the lateral ear vein.

Armour's thyroid extract was used in all experiments, the tablets being given by mouth. Five animals received 2 grains per day for a period varying between 36 and 51 days, two received 4 grains per day for 34 days, two 6 grains per day for 21 days, two the same amount for 34 days, two 8 grains per day for 16 days and one the same amount for 21 days. Following this prolonged medication the animals were placed in the same posture as in the control experiments and 0.2 cc. of pitressin was injected intravenously while the electrocardiogram was being taken. The electrocardiogram in each case was then compared with the first or control record.

In those cases in which the animal did not die following the injection of 0.2 cc. of pitressin it was killed by cerebral concussion. After bleeding the animal the heart was excised and sent to the pathology laboratory for study.

RESULTS

Some of the modern text-books of physiology (9) still adhere to the old observations that the normal rate of heart beat of the dog is 100-120 beats per minute and that of the rabbit 150. Cloetta (10) published a few isolated observations to the effect that the heart rate in rabbits varied between 210 and 240 beats per minute. In 24 control animals we found the heart rate of the rabbit to vary between 180 and 285 beats per minute for an average of 236 beats. In some experiments the animals were kept quiet for more than an hour without showing a change in the heart rate.

In the 14 animals treated with thyroid extract the new control rate varied from 288 to 440 beats per minute with an average for all the animals of 360 contractions per minute. The original controls for these same animals before the administration of thyroid averaged 233 beats per minute, the smallest increase following thyroid feeding being 60 beats per minute. It is very evident from our results that the average normal heart rate in rabbits is much higher than given in text-books of physiology and that thyroid extract medication causes a tachycardia amounting in our cases to more than 54 per cent.

Our results confirm Clark's findings (5) i.e., pitressin in 0.3 cc. doses, injected intravenously in normal, healthy rabbits, is not very toxic. In our experiments 0.2 cc. was injected in each of 12 normal animals and 0.3 cc. pitressin was injected intravenously 70 times in 61 animals without a single death. Changes in the heart were noted in the E.C.G. taken, although none of these were of a serious nature. In 12 animals 0.5 cc. and, in 3, 1 cc. of pitressin was injected intravenously. Marked constriction of the pupils, defecation and marked electrocardiogram changes of the heart were recorded although no deaths occurred.

In the 14 animals which had been treated with thyroid extract, injections of 0.2 cc. of pitressin caused three to die from ventricular fibrillation, one to show a period of temporary ventricular fibrillation from which it recovered and the others to show pathological changes in the electrocardiogram far in excess of those produced in the early controls by the injection of 0.3 cc. of pitressin.

Figures 1 and 2 are electrocardiograms taken from two animals and

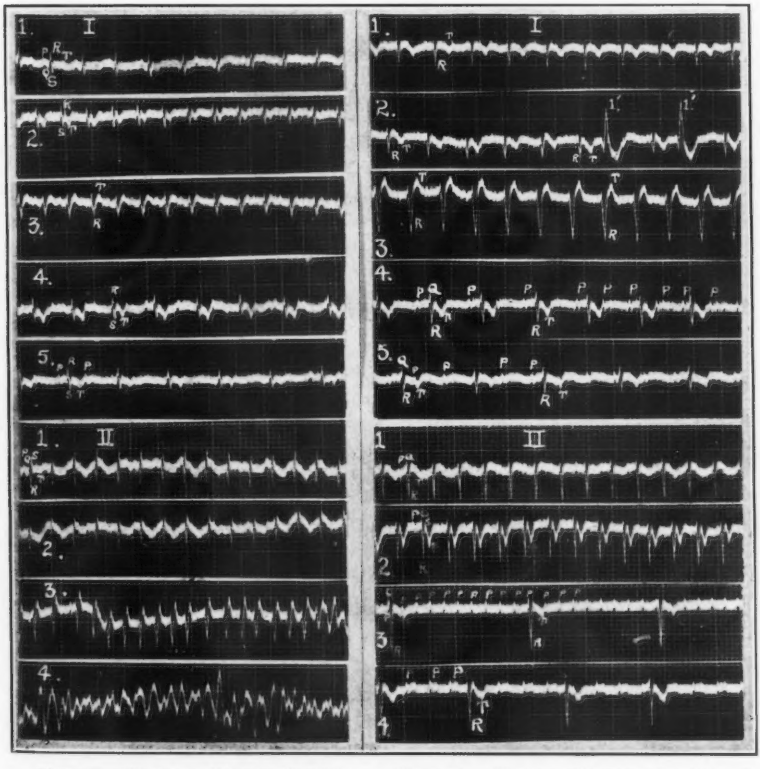

Figure 1. Electrocardiogram of rabbit. I, before the administration of thyroid extract and II, after the extract. Weight of heart, 8 gm.

Figure 2. Electrocardiogram of rabbit. I, before thyroid extract and II, after thyroid. Weight of heart, 12 gm.

are typical of all the results. In Figure 1 a 3.2 kg. rabbit was used. In it, in I, at 1 is illustrated the normal electrocardiogram in which the heart rate was 240 beats per minute. Between strips 1 and 2, 0.3 cc. of pitressin was injected intravenously. There can be seen in 2 an inversion and an increase in the depth of the T-wave. In 3 there is complete inversion of the diphasic R-wave which can be seen best in 4. In 5 complete A-V block is recorded from which the animal rapidly recovered.

Figure 1, II is a series of segments of electrocardiograms of the same animal almost two months later, during which time 95 grains of thyroid extract had been administered by mouth. The new control heart rate at this time as seen in II, 1, is 390 contractions per minute and there is normally an inversion of the T-wave. Between record 1 and 2, 0.2 cc. of pitressin was injected intravenously. Although this animal had tolerated 0.3 cc. of this same drug without much cardiac disturbance as seen in this figure at I, after the feeling of thyroid extract two-thirds the original dose caused ventricular fibrillation as seen in II, 4, from which the animal did not recover.

In Figure 2, a 3.5 kg. rabbit was used. The control heart rate as seen in I, 1, was 220 beats per minute, which was increased by thyroid feeding to 320 beats per minute as seen in II, 1. Between I, 1 and I, 2, 0.3 cc. of pitressin was injected intravenously. Premature contractions, 2, 1′, a displaced pace maker, 3, and complete A-V block 4, resulted from this injection. These changes are, however, very much less than those caused by two-thirds the amount of this dose when administered in the same animal following 98 grains of thyroid extract fed over a period of 39 days. These results appear in Figure 2, II. Many auricular contractions are seen, in 4, with only an occasional ventricular complex. There are also high branching T-waves as seen in II, 2, indicative of marked vasoconstriction of the coronary vessels.

Our results agree with Clark's finding that there is markedly increased sensitivity in the rabbit's heart to pitressin following a variable period of administration of thyroid extract.

Pathology. Although Hoskins (11), Herring (12), Hewitt (13), Hashimoto (14), Goodpasture (15), Takane (16) and Gruber, Olch and Blades (7) showed either gross or microscopic changes in the hearts of rabbits, rats, cats and guinea pigs fed thyroid extract, Cameron and Carmichael (17) and Rake and McEachern (18) found negative results in all animals thus treated. Because of this disagreement we have repeated our observations. In 13 of the 14 animals examined varying degrees of myocardial changes were noted (see Fig. 3). In only one case was the heart considered absolutely normal. The changes noted were moderate degeneration of the muscle fibers, as shown by irregular density and granular cytoplasm, slight edema between the muscle fibers and usually some infiltration with monocytes. Capillary dilatation was also encountered and in some cases fibrosis.

These changes are best illustrated in Figure 3 (1, 2 and 3). In 1, there is seen marked degeneration, necrosis and autolysis of the muscle fibers. A moderate monocytic infiltration is also present. In other areas as seen in the same heart, 2, there is active fibroblastic proliferation in areas of myocardial degeneration. In 3, the field shows the type of myocardial fibrosis which results from the healing of such lesions as seen in 1 and 2.

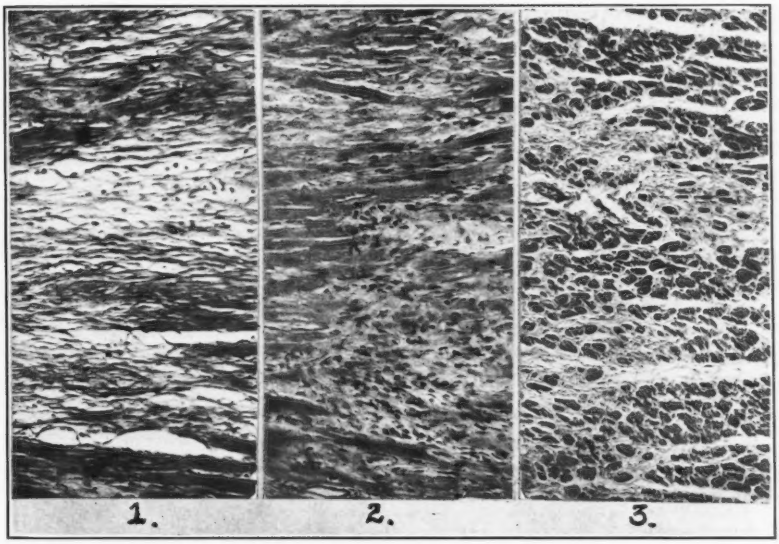

Figure 3. Photo-micrographs of heart muscle showing abnormal changes. See text.

SUMMARY AND CONCLUSIONS

1. The normal cardiac rate in 24 rabbits varied between 180 and 285 beats per minute with an average of about 230.

2. Thyroid extract given by mouth increases the rabbit's heart rate. In our series of 14 animals it was increased from an average of 233 to an average of 360 contractions per minute.

3. Thyroid extract administered orally to 14 rabbits over a long period of time increased the sensitivity of the heart to pitressin.

4. Ninety per cent of the hearts of animals fed thyroid extract showed muscle damage.

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THE RELATION OF DIET AND SERUM-CALCIUM TO TETANY IN THE PARATHYROIDECTOMIZED RAT

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It is well known that the response of the albino rat to parathyroidectomy varies more than that of other mammals. Dogs and cats, for instance, almost invariably die of tetania parathyropriva unless therepeutic means are used to preserve them; but most reports on rats show that some of these animals may survive for weeks or months after they have been deprived of their parathyroids. This fact makes the rat an excellent animal for the study of the underlying causes of the syndrome, since it affords the opportunity for a comparative study of tetany and no-tetany cases.

The two explanations most frequently given for the failure of some animals to develop tetany are these: First, that accessory parathyroids are present in all those which do not show symptoms of tetany; second, that the calcium-intake in the diet of surviving animals is sufficient to offset the loss of this element from the blood stream which is the usual result of the operation. The second explanation has been widely accepted among biochemists, and is expressed as follows by Shelling (1932): "In the study of parathyroid tetany the calcium and phosphorus of the diet are the determining factors for the development or non-development of tetanic symptoms."

The author of this paper has already expressed doubts as to the validity of either of the above explanations of the absence of tetany (Hoskins, 1931). In the experiments the results of which are published in the paper just cited, very careful microscopic search was made for accessory parathyroids and for glands overlooked in the operation. In that series, as in a study of the frequency of accessory parathyroids (Hoskins and Chandler, 1925) it was found that such tissue is present in about 5 per cent of the rats examined, while 50 per cent of those parathyroidectomized survived without any discoverable trace of parathyroid tissue.

The statement that diet is not entirely responsible for the variability in response to parathyroidectomy was based on experiments with a considerable number of litters of rats which had been raised under identical conditions from birth to the time of operation. The average susceptibility within such homogeneous groups was 50 per cent, and this was taken as an indication that tetany depends partly on endogenous factors. However, the diet used at that time consisted of bread, lettuce, cabbage, meat, and fish; and the combinations of these foods given on different days varied. It seemed possible that individuals in the same cage might, by making different selections, provide for themselves different amounts of calcium

and phosphorus. In order to eliminate this source of error, it was decided to use diets of known chemical constitution, in a paste form from which selection was impossible. The original intention of the present study was not, in fact, to investigate further the relation of diet to tetany, but to begin a search for those endogenous factors which control or contribute to the appearance of tetanic symptoms. As a first step it seemed advisable to find standardized diets, the effects of which could be accurately predicted. From a perusal of the biochemical literature it seemed that it should be possible to select two rations which would guarantee a high and a low percentage of tetany, respectively. My lack of success in doing this has led to certain conclusions with regard to diet, serum-calcium, and tetany which are given in the following pages.

MATERIAL AND METHODS

1. *Animals.* Rats were obtained for this study from various sources: From the Biochemical Department, Yale University; from the Breeder Company, New York; from the Department of Anatomy, University of Pittsburgh; and from the Department of Physiology, University of Chicago. All those described in this paper were parathyroidectomized at 55 to 60 days of age. Unless otherwise stated, they were of weights equal to or above the figures given as normal in the Wistar Institute tables (Donaldson, 1924).

2. *Diets.* The "Stock diet" and the diets lettered from A to D, inclusive, were based on the work of Kramer and Howland (1932). The foundation of this is as follows:

	Grams
Rolled oats	400
Gelatin	100
Dextrin	410
Wheat gluten	70
NaCl	10
KCl	10

To this, varying amounts of $CaCO_3$ and KH_2Po_4 were added. The amounts of Ca per 100 grams and the Ca/P ratios of these diets are given in Table I. Diet E is from Shelling's paper (1932). It is his diet I-K, and contains the following elements:

	Grams
Whole wheat flour	400
Casein	100
Cornstarch	325
Wheat gluten	50
Butter	50
Olive oil	40
NaCl	20
KCl	15

In diet M a return was made to the old mixed diet of bread, lettuce, etc., on which rats in former years had developed tetany to about 50 per cent. This diet was used again, in spite of the disadvantages mentioned above, when the weighed diets failed to yield a satisfactory percentage of tetany. The average Ca and P of diet M, as given in Table I, were estimated from Sherman's tables (1918).

3. *Operations.* The technique of parathyroidectomy used in these operations was the same as that described by Overholser (1929).

4. *Calcium determinations.* Blood samples were obtained by anesthetizing the rats with ether, opening the pericardial cavity, and drawing blood with a syringe from the right ventricle while the heart was still beating. Serum-calcium determinations were made by the Kramer-Tisdall method.

5. *Check on completeness of operation.* Microscopic examination of the thyroid and immediately surrounding tissues was made in all cases in which the blood-calcium was found to be 6.5 mg. per 100, or over, and in all cases in which the blood-calcium was not determined.

RESULTS

1. *The relation between diet and the percentage of tetany.* In two of the diets used in this study the Ca/P ratio was 1.66. Rats fed on diets having this Ca/P ratio appear to be immune to the immediate effects of parathyroidectomy, for no symptoms of tetany developed in any of the 57 cases recorded. In diets A, C, and D the Ca/P ratio was reduced to 0.83, 0.5, and 0.045, respectively. Of 20 rats on these diets only 3 (on diet A) developed tetany after parathyroidectomy. On diet E, which had a Ca/P ratio of 0.025, only 16 of 44 rats died of tetany. An attempt was made to reproduce the 50 per cent mortality of former years, when rats were fed on a mixed diet (M). The Ca/P ratio of this mixed diet was estimated to range from 1.6 to 0.06, with an average figure of 0.55. The mortality on this diet in the present series was 16 of 44 cases, a trifle higher than that obtained on the Ca-free ration.

The conclusion is that an adequate Ca/P ratio in the diet protects parathyroidectomized rats against tetany, but a gradual reduction of this ratio does not bring about a corresponding increase in the percentage of animals suffering from tetania parathyropriva. Thirty per cent mortality occurred on a diet with a Ca/P ratio of 0.83, and this figure was not exceeded on a diet which was almost devoid of calcium. It appears that the percentage of tetany in parathyroidectomized rats cannot be controlled with accuracy by variations in the diets on which the animals are fed.

2. *Diet and serum-calcium.* In determining the effect of diet on serum-calcium, diets B, E, and M were used. None of these caused a significant alteration of the blood-calcium of the control rats. Parathyroidectomized animals on diet B had an average serum-calcium of 7.9, as compared with a figure of 10.1 for their controls. Among parathyroidecto-

mized rats on diet M the average serum-calcium at 24 hours was 5.8, the greater drop in blood-calcium corresponding to the reduction of the dietary Ca/P from 1.66 to 0.55. Further reduction of the Ca/P ratio of the diet does not, however, induce a correspondingly greater fall in serum-calcium after parathyroidectomy. On diet E, which was almost devoid of calcium and had a Ca/P ratio of only 0.025, the average serum-calcium of operated rats was 5.3 mg. per 100 cc., only 0.5 mg. below the figure for those on diet M.

The conclusion is that the level of the serum-calcium does not follow the reduction of the dietary Ca/P beyond a certain point. It can be forced down to an average of about 5 mg. per 100 cc., but does not go lower on a diet which is practically calcium-free.

TABLE I

		No. Cases	Range of Serum-Ca.	Average Serum-Ca.
Group I	N. T.	9	8.0—9.4	8.6
	T. A.	0	.....	...
	M. S. T.	0	.....	...
	F. T.	0	.....	...
Group II	N. T.	23	6.1—7.9	6.8
	T. A.	6	6.1—7.7	6.6
	M. S. T.	3	6.2—7.1	6.6
	F. T.	0	.....	...
Group III	N. T.	11	4.0—5.7	4.9
	T. A.	12	4.2—5.6	4.9
	M. S. T.	8	3.6—5.5	4.7
	F. T.	4	3.9—5.6	4.6

3. *Diet and tetany.* From the above results it may be deduced that dietary Ca/P exercises a limited and somewhat uncertain effect on the serum-calcium levels and the percentages of tetany obtained after parathyroidectomy. It might be supposed that the development of tetany does, in fact, depend on the serum-calcium alone, and that the failure of diet E to product more than 30 per cent mortality was due to its ineffectiveness in lowering the serum-calcium. A somewhat different view of the actual relation between tetany and serum-calcium is suggested when the figures are arranged as in Table I. Here a different grouping has been used, since it seemed advisable to take into account varying degrees of tetany. Animals classed as "No tetany" cases are those in which no tremors were observed at the time of killing. In the next group ("T.A.") are those which were apparently normal when taken from the cage, but which developed symptoms of tetany when anesthetized and bled. The third group ("M S T") includes all cases of rats which were killed during mild but spontaneous attacks of tetany. In the last column are animals which were in tetany so severe that it would undoubtedly have proved fatal if they had not been killed by ether. The table is divided horizontally into three groups on the basis of the serum-calcium determinations. In the first division are 9 animals having a serum-calcium well within the normal range, and no symptoms of tetany. In the second group tremors under anesthesia

and mild attacks of spontaneous tetany appeared. For this group the range in serum-calcium level is 6.1 to 7.9 with an average figure of 6.7. The cases of tetany of both degrees of severity are as widely distributed throughout the series as are the instances free from tetany, and the average figures for all three classes are almost identical. In other words, it is impossible to subdivide the group in such a manner as to correlate tetany with serum-calcium. The same lack of close correlation between serum-calcium and tetany is apparent in the third group. Here, it is true, a smaller proportion of rats was free from tetany, but the distribution and the average of the serum-calcium figures of this group afford no explanation of the variation in susceptibility to tetania parathyropriva existing within it.

In addition to the cases listed in Table I, 18 rats of the entire series of experiments died of tetany during the night following parathyroidectomy, and no blood samples could be taken from these. It is possible that some, or all, of these animals had serum-calcium levels lower than those presented in the table. In that case we should have a fourth group, in which the serum-calcium would be 3.9—, and death the universal result of the operation. The existence of such a group is, however, entirely a matter of conjecture.

The data on the relation between serum-calcium and tetany may be summarized as follows: There is an increase in the percentage of cases developing tetany and in the severity of the symptoms, which is parallel to the increase in the calcium-drop following parathyroidectomy. Animals which maintain a normal, or nearly normal, calcium level are not subject to tetany. If the serum-calcium falls to a point below 7.9 tetany may or may not occur. With values between 7.9 and 4.0 every case of tetany in these records can be matched in its calcium level with another case in which no symptoms were to be observed at the time of killing. It is thus apparent that while serum-calcium is a contributing factor in the syndrome, it must work in connection with some other element in the situation in the development of tetany.

TABLE II

Season	Av. Weight*	N. T.	T. A.	M. S. T.	F. T.
April	+38	3	2	..	1
April	+14	2	3	..	..
April	+26	4	1	..	..
April	+12	2	2	..	..
November	+19	7	..	..	..
March	+ 8	1	..	3	1
May	−19	..	..	1	7

*Expressed in terms of grams above or below the weight given in the Wistar tables for rats of the corresponding age and sex.

4. *Nature of the factors influencing the development of tetany.* Many factors besides the blood calcium have been thought to influence the susceptibility of rats to tetania parathyropriva. It is known to vary with the age and weight of the animals; and climate, season, and nervous condition have been suspected of playing a part in the development of tetany.

Other dietary factors than calcium and phosphorus have been blamed for variations in mortality, also. In Table II is presented a series of experiments in which all external factors were carefully controlled. This gives the results of parathyroidectomy on 7 different groups of rats, all of which were kept under the same conditions of sunlight, ventilation, etc., and fed on a standardized diet (E). All these animals were parathyroidectomized at 60 days of age. The two factors which did vary somewhat among the groups, namely, their weights and the dates of operation, are listed in the table. It will be seen that seasonal differences do not account for the varying percentages of tetany among the 7 groups. Among the 14 rats which were parathyroidectomized between the 18th and the 25th of April, 8 showed no signs of tetany and the remaining 6 only the mildest form of symptoms. But in the group operated on the 8th of May, all the rats had spontaneous tetany, which ended fatally in 7 of 8 cases.

The seventh group is the only one in which the average weight of the rats was below the normal, and also the only one in which a large percentage of mortality occurred. It is probable that this correlation is significant. The weight deficiency, however, must be regarded as an expression of some internal disturbance rather than as the result of a dietary deficiency. That animals on diet E can maintain normal weights is shown by all the other groups in the table.

A further demonstration of the variation in susceptibility which is possible under identical external conditions is given by two groups of operations which were performed at the end of the series and are not included in the tabulations. Two litters were born in our colony on the same day, and were put on diet E at the same time. All were parathyroidectomized in one morning, with the following result: The five members of one litter were found dead on the next day, while none of the other litter has succumbed. Of the second lot, one had no symptoms of tetany (serum-Ca. 4.7), two developed symptoms upon bleeding, and two had fairly severe spontaneous tetany. It is evident that the different reaction of the two litters must have been the result of internal rather than external differences.

It appears, then, that some rats have a constitutional resistance to tetany, while others are not so protected. Experiments were made with the purpose of ascertaining whether this constitutional resistance was better developed in some breeds of rats than in others. Animals were secured from various sources, and parathyroidectomized under standardized conditions. Rats from the colony at Yale University proved very resistant to the effects of the operation, and those coming from the Breeder Company failed to develop tetany in satisfactory numbers. The author learned that the animals in the colony at the University of Chicago had a high susceptibility, and through the kindness of Professor Barnes was able to operate on 20 rats sent to her from there. Their response to the operation was not, however, different from that of other stocks we have tested. Without a thorough comparison of Professor Barnes' methods with our

own, it is impossible to say why our results are so different from his; but the experiment indicates that a high susceptibility to tetany is not a permanent inherent quality of one breed or another of rats.

DISCUSSION

It will be seen from the foregoing pages that an unexpected difficulty has been encountered in studying the causes of parathyroid tetany. It was supposed at the outset of the series that, given a standardized diet with sufficiently low calcium content, animals in tetany could readily be secured for study. The work planned was a histological study of the endocrines of rats in tetany, but all that has been accomplished is the bringing out of certain relations between diet and tetany.

The point that calls for explanation is why so many animals failed to develop tetany. Two of the three diets used have, at other times, been correlated with much higher mortality. Diet M was used in this laboratory from 1925 to 1930, during which time 50 per cent of the parathyroidectomized rats died of tetany. Diet E, as used by Shelling, was reported to produce even greater mortality. He states that most of the rats placed on this ration for a few days prior to the operation developed severe tetany and died within 12 to 24 hours. This, nothwithstanding the fact that the rats used by Shelling were 6 months old and should have been considerably less susceptible to tetany than those at 60 days of age selected for our experiments.

One cannot resort to the obvious explanation that the survivors in this series were incompletely parathyroidectomized, in view of the fact that many of them showed a marked drop in serum-calcium. If one doubts the completeness of a microscopic search for parathyroid remains, the blood-calcium values offer proof that these operations were successful.

The total result of the experiments may be expressed as follows: Alterations in diet may force serum-calcium down to values between 4 and 5 without producing symptoms of tetany in all cases. We are thus obliged to qualify our acceptance of the importance of serum-calcium as the sole factor in the production of tetany. Obviously it is one factor, since animals with normal or nearly normal serum-calcium levels do not exhibit tetanic symptoms, but there must be contributing factors to the syndrome. The nature of these is not clear. From the results of carefully controlled experiments they appear to be primarily constitutional. One cannot, of course, exclude the possibility that differences in external conditions may act on such constitutional factors. Changes in environment, for instance, may alter the endocrine balance without affecting the serum-calcium response to parathyroidectomy.

In considering the relation of serum-calcium and other factors to tetany, it is of interest to note that the rat has, apparently, some internal mechanism which compensates for the loss of the parathyroid. Throughout our experiments it has been observed that death from tetany usually occurs within 48 hours after parathyroidectomy. Animals which survive

this critical period may live for months, their final symptoms being those of weakness and collapse rather than of tetany. It is possible that in the groups of rats with low serum-calcium the difference between animals which develop tetany and those which survive lies in the speed with which the compensatory mechanism comes into action. In some cases it is, at the time of operation, ready to function during the critical period; in others, it is not prepared, and the delay causes tetany. According to this view, tetany is prevented by one of two factors; namely, a high serum-calcium or an active compensatory mechanism. The operated animals would thus fall into four groups:

1. High serum-Ca, active compensatory mechanism, no tetany.
2. High serum-Ca, inactive compensatory mechanism, no tetany.
3. Low serum-Ca, active compensatory mechanism, no tetany.
4. Low serum-Ca, inactive compensatory mechanism, tetany.

As to the nature of this compensatory mechanism we know little at the present time. It apparently gains in activity after puberty, but is poorly developed in sickly, underweight animals. It is hoped that studies now in progress in this laboratory, which have as their object the understanding of the relations of the other endocrines to parathyroid tetany, may throw some light on the subject.

SUMMARY

The study is based on the results of over 160 operations on albino rats of 60 days of age. Seven diets were used in which the Ca/P ratio ranged from 0.012 to 1.66. It was found that the higher Ca/P ratios protected rats from tetany by preventing a considerable drop in serum-calcium. If diets with lower Ca/P ratios were used, the serum-calcium of the operated animals dropped, and some cases developed tetany. The mortality was never greater than 30 per cent. In groups with low serum-calcium values, tetany could not be correlated at all closely with the blood calcium level, and appeared to depend on some endogenous factor. The conclusion is that while a low serum-calcium is a necessary part of the tetany syndrome, the development of symptoms depends also on other endogenous factors the nature of which is not known.

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STUDIES IN THROMBO-ANGIITIS OBLITERANS (BUERGER) X. REDUCTION IN BLOOD VOLUME FOLLOWING BILATERAL OOPHORECTOMY

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One phase of our investigation of thrombo-angiitis obliterans has centered upon determining the significance of the low blood volume present in this condition. One of the puzzling facts about thrombo-angiitis obliterans is its rare occurrence in women. The theories that have been held to account for this immunity have included, first, differences in the habits and exposure of the two sexes; second, the possibility of a sex-linked hereditary factor; and third, a biological difference which protects women against the disease. Investigating the last of these theories, it seemed logical to assume that the ovaries might be responsible for this immunity. Certain known facts led us to believe that changes in the blood were produced by oophorectomy. For instance, women who have had both ovaries removed occasionally suffer from repeated attacks of phlebitis in the lower extremities during the years following the operation. It seemed possible that some alteration in the blood was responsible for this. Chemical changes in the blood after bilateral oophorectomy have been reported by other investigators (1, 2), but none have included study of the blood volume. For these reasons we decided to make a simultaneous study of the chemistry and blood volume in women and in female animals from whom both ovaries had been removed. We selected cholesterol, fibrinogen and viscosity as factors which might have a bearing upon the greater tendency to thrombosis.

CLINICAL MATERIAL

The patients used for this study were selected from Dr. Frank's Service on the Wards and Out-Patient Department of The Mount Sinai Hospital and from our private practice. This included 25 patients (Chart I) who had had a surgical removal of both ovaries and the uterus, and a control group of 21 patients (Chart II). In addition, a number of female cats were subjected to bilateral oophorectomy. The volume and chemical composition of the blood were determined and checked on the cats before operation, and repeated every month after the operation. The data are presented in case of those animals which survived for a sufficiently long time to allow repeated determinations to be made.

METHODS

Blood volume determinations were made by the dye method of Lampe, modified by Rosenthal, as described by Silbert, Kornzweig and Friedlander (3). Fifty normal individuals gave an average of 80 to 85 cc. per kilogram body weight by this method. Similar results were obtained by other investigators. Cholesterol determinations were made by Bloor's method, and in normal individuals the figures range from 150 to 200 mg. per 100 cc. The Wu method was used to determine fibrinogen, and the normal range is 2 to 2.5 mg. per 100 cc. The Hess apparatus was used in determining the viscosity of the whole blood. Normal figures are 4 to 5. The blood volumes in animals were determined by utilizing the external jugular veins while under mild ether anesthesia. These veins are relatively large and with a little practice can be readily entered so that accurate determinations are possible.

CHART I

BLOOD CHANGES FOLLOWING BILATERAL OOPHORECTOMY IN 25 WOMEN

Patient	Weight	Post-Operative Interval	Age	Basal Metabolism	Blood Volume cc. per Kg.	Viscosity	Cholesterol	Fibrinogen
		Months						
T. A.	145	1½	27	....	76	....	200	3.
F. L.	131	1½	37	....	70	5.6	210	2.6
R. T.	152	6	34	—14	61	5.	235	3.4
S. F.	142	11	49	....	66	8.	220	3.3
C. B.	151½	14	38	....	71	5.6	250	2.5
M. S.	134	11	38	....	54	5.5	210	2.9
M. B.	164	16	46	....	50	5.4	165	2.6
R. H.	110	18	42	....	73	...	200	3.2
A. S.	140	18	42	....	53	5.	210	3.
B. T.	145	20	40	....	68	...	210	3.
M. E.	195	21	42	....	56	5.2	250	2.9
E. U.	152	22	34	....	60	6.	210	2.9
J. J.	225	25	50	....	64	6.	210	2.8
C. K.	148	25	45	....	51	6.	210	2.8
C. T.	168	24	38	....	53	5.8	250	3.
A. P.	168	32	38	....	47	...	310	4.
L. P.	144	34	40	....	52	6.4	220	3.
P. S.	171	32	49	....	51	5.6	250	3.2
B. R.	156	49	46	....	62	7.	...	...
S. O.	124	55	34	....	59	6.	260	3.
H. M.	156	140	38	—25	63	6.	250	2.6
M. S.	141	60	42	—13	66	5.4	190	2.8
H. P.	156	72	47	—13	58	7.	300	3.
R. A.	138	108	50	—21	54	5.	...	...
Average...	152	...	41	—17	59	5.9	239	2.9
*F. P.	117	15	41	+47	85	5.	180	2.

*Not included in average because of presence of hyperthyroidism.

RESULTS

Chart I presents the blood volume, cholesterol, fibrinogen and viscosity in 25 patients after the surgical removal of both ovaries and uterus. It will be noted that the average blood volume in these cases shows a reduction of about 25 per cent from the normal. This change becomes more striking after the first few months. Coincident with this there is an elevation of the cholesterol and fibrinogen of the plasma and an increase in viscosity of the blood. Since cholesterol figures are readily altered by physiological changes they should not be given too much weight. How-

ever, it is interesting to note that in our cases there is a consistent tendency for these figures to be higher and they are supported by a diminished blood volume. A normal blood volume was obtained in only one patient who had had a bilateral oophorectomy.* Here there was a definite clinical picture of hyperthyroidism with a basal metabolism of plus 47 per cent. The normal blood volume in this case is probably due to the increased thyroid activity. Silbert and Friedlander have reported that the low blood volume in thrombo-angiitis obliterans cases can be restored to normal by the administration of thyroid extract (4).

CHART II

BLOOD VOLUME AND CHEMISTRY IN A CONTROL SERIES OF 21 WOMEN

Patient	Weight	Condition	Age	Blood Volume in cc. per Kg.	Viscosity	Cholesterol	Fibrinogen	Duration in Mos.
I. B.	150	Natural menopause.....	53	71	6.	...	...	34
F. S.	138	Natural menopause.....	46	88	5.	190	2.5	11
C. H.	138	Natural menopause.....	44	83	4.4	170	2.	14
R. K.	146	Natural menopause.....	45	83	4.5	140	2.	40
R. N.	163	Natural menopause.....	54	77	5.	...	...	..
S. W.	142	Natural menopause.....	40	72	4.5	180	2.	..
R. S.	134	Natural menopause.....	..	93	...	190	2.2	..
E. D.	137	Radiotherapy menopause.....	37	71	6.	190	2.6	12
R. C.	149	Radiotherapy menopause.....	45	80	5.	...	...	46
L. G.	127	Radiotherapy menopause.....	..	80	...	200	2.5	..
L. S.	115	Radiotherapy menopause.....	..	73	...	200	2.4	..
H. N.	137	Recurrent amenorrhea.....	34	75	6.	180	2.	48
S. S.	120	Hysterectomy and left oophorectomy.....	37	83	6.	160	2.2	48
B. G.	102	Right salpingo-oophorectomy.	21	89	5.5	180	1.9	2
S. M.	192	Vaginal plastic.....	42	72	4.4	...	...	44
M. S.	110	Vaginal plastic.....	33	90	4.	150	1.9	20
Z.	155	Vaginal plastic.....	38	75	5.	150	2.	16
S. J.	125	D. and C.....	37	77	4.5	200	2.	4
P. K.	...	D. and C.....	48	77	5.	200	2.5	4
R. G.	125	D. and C.....	42	88	5.	180	2.	8
M. S.	106	Pre-operative for pan-hysterectomy.....	..	89	...	200	2.2	0
Average.	135½	.....	41	80.2	5.04	182	2.16	..

In Chart II corresponding studies of a miscellaneous control group are presented. All the blood volume figures are within normal limits and the cholesterol, fibrinogen and viscosity are correspondingly lower. This group includes 11 patients with normal menopause and artificial menopause produced by radiotherapy. It would appear, therefore, that some substance capable of influencing the blood volume is produced by the ovaries even after other physiological functions have ceased. Further investigations are being carried on in this direction.

We have reproduced these results in animals in order to study the biological factors involved. In Chart III are summarized the results obtained in a series of six of the cats subjected to operation. In order to simplify the table, only the first and last determinations are given, but in practically every case these were made each month and showed consistent

*We express our appreciation to Dr. Frank for his interest and cooperation in this work.

CHART III

BLOOD CHANGES FOLLOWING BILATERAL OOPHORECTOMY IN 6 CATS

No.	Date	Blood Volume in cc. per Kg.	Plasma Per Cent	Weight in Kg.	Cholesterol	Fibrinogen	Operation
69	6/19/33	73	60	3.5	70	1.8	Hysterectomy and bilateral oophorectomy.
	1/ 5/34	48	56	3.4	100	2.0	
39	6/18/33	74	62	2.6	90	2.	Hysterectomy and bilateral oophorectomy.
	12/28/33	50	57	3.2	110	2.	
37A	4/12/33	64	60	2.7	75	2.	Bilateral oophorectomy.
	12/19/33	42	68	3.3	110	2.1	
37	9/ 7/32	70	64	2.5	70	2.	Bilateral oophorectomy.
	2/28/33	40	60	3.3	120	2.5	
29	9/27/32	60	64	2.4	70	2.	Left oophorectomy.
	5/ 9/33	68	61	2.2	80	1.9	
5A	5/ 5/33	65	58	2.7	90	2.1	Bilateral oophorectomy.
	1/ 2/34	50	60	3.	110	2.	

changes. Many other animals were operated upon but the mortality due to intercurrent diseases interfered with a sufficiently long period of observation in many of them. In general it may be said that all these cats showed a consistent tendency toward a reduction in blood volume after bilateral oophorectomy. It is interesting to note that in one animal, No. 29, in which only a single ovary was removed, there was no reduction in volume.

DISCUSSION

Striking changes in the blood volume (3) and chemistry (5) in the majority of individuals with thrombo-angiitis obliterans were reported by us in 1930 and 1931. Whether such alterations are present before the development of the condition or whether they result from the presence of the disease has not yet been established. We have now found that similar changes in the blood are produced in women by bilateral oophorectomy. Thrombo-angiitis obliterans was not observed in any of the women following castration, but none of these individuals were smokers. Thrombo-angiitis obliterans does not occur in non-smokers, and therefore one could not expect the disease to develop in this group. Gynecologists avoid removing both ovaries in young women if at all possible, and always try to leave a small fragment of an ovary if no more can be saved. Therefore opportunities are not very frequent to study the peripheral circulation in young women who have had both ovaries completely removed and who are smokers. If evidence of thrombo-angiitis obliterans were observed in such individuals in higher than the expected incidence the importance of the ovaries as a protective mechanism against this disease would be established. It would also suggest that the reduced blood volume may precede the development of the disease.

SUMMARY

1. A study of the blood in 25 patients following surgical removal of both ovaries shows a consistent reduction in blood volume of about 25 per

cent. Coincident with this the cholesterol, fibrinogen and viscosity are increased.

2. Similar changes are demonstrated in a series of female cats subjected to bilateral oophorectomy.

3. A corresponding study of a control group, including 11 patients with artificial and natural menopause, shows no changes in blood volume or chemistry.

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EXPERIMENTAL HYPERTROPHY AND ATROPHY OF THE PROSTATE GLAND

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For some years we have been studying the etiology of prostatic hypertrophy. One of the first steps in our studies was the investigation of a substance, androtin, which is secured from the urine of males, which produces comb growth in capons and which has all the physiologic properties of a hormone which has been extracted from the testes (1). However, we soon found that this hormone alone failed to maintain a castrated animal in a completely normal state, and hence failed to explain all the endocrine functions of the testes. Androtin maintains the size of the secondary sex glands but fails to correct the hypertrophy of the pituitary which results from castration.

These experimental findings prompted us, two years ago, to postulate the existence of a second internal secretion from the testes, one property of which is the depression of pituitary activity (2). This hypothetical substance, therefore, has been called inhibin. We have previously demonstrated that androtin will cause prostatic hypertrophy in normal rats. The hypothetical inhibin would be expected to cause prostatic atrophy.

Accumulating evidence, both in our laboratory and in the experiments of other workers in this field, seems to argue more and more in favor of the existence of inhibin. Some of the evidence cannot as yet be adequately explained, but we are hopeful that as the work progresses some of these apparently contradictory results will be elucidated.

This paper includes a general summary of our studies. The figures in the tables represent average values and standard deviations for the series. Detailed accounts of the various investigations, including protocols of the experiments and the histological findings, are to be reported in several subsequent publications.

Martins and Rocha (3) have been led to the conclusion that the testes secrete a substance that depresses pituitary activity. Their experimental work was done on parabiotic animals—that is animals which are united surgically so that their abdominal cavities are continuous and chemical substances can pass from one to the other. They found that if one of two males thus united is castrated, there is definite stimulation of testicular function, which results in prostatic hypertrophy in the normal partner. This is interpreted as due to hyperactivity of the pituitary gland of the castrated animal. Martins and Rocha then showed that the implantation of testicular mush into the castrated partner prevented the hypertrophy of the prostate in the other partner. Their explanation was that the testes contain some substance which depresses pituitary activity.

We repeated these experiments on a larger series of parabiotic animals, injected rat testes mush into the castrated partner, and obtained results identical with those of Martins and Rocha. Since it was impossible to perform an extensive series of experiments using preparations from rats' testes, we repeated the experiments, using a preparation of bulls' testes, and again obtained identical results.

These experiments indicated merely that there was a substance in the testes which depressed the activity of the pituitary gland. In order to determine whether or not the fat-soluble male sex hormone, androtin, might cause the effect, we repeated the experiments, using this substance instead of testes mush and found that the effects produced were similar. With androtin, the size of the secondary sex glands of the castrated partner was maintained and in addition those of the normal partner remained apparently normal.

These experiments on parabiotic animals, the results of which are shown in Table 1, indicate that the injection into the castrate of testes mush or of the oil-soluble testicular hormone from male urine prevents the prostatic hypertrophy which ordinarily occurs in the normal partner of a normal-castrate parabiotic union. We can offer no explanation for the fact that androtin produces the same effect as whole testes.

However, in Martins and Rocha's experiments as well as in ours, it was obvious that the testes mush could not have contained more than a trace

TABLE I

EFFECTS OF INJECTION OF VARIOUS TESTICULAR EXTRACTS IN PARABIOTIC RATS

	Original Average Weight	Final Weight	Days	Mean Ratios of Organ Weight to Body Weight and Standard Deviations of the Means				
				Testes	Epididymis	Seminal Vesicles	Ventral Prostate	Dorsal Prostate
16 Normal controls	gm.	gm. 191	..	1.131 ±0.048	0.337 ±0.015	0.248 ±0.026	0.150 ±0.012	0.054 ±0.006
6 Castrate controls	207	200	22	....	....	0.043 ±0.002	0.015 ±0.001	0.010 ±0.0005
8 Untreated parabiotic pairs— (a) Normal partners	190	171	20	1.326 ±0.143	0.418 ±0.042	0.671 ±0.113	0.263 ±0.030	0.139 ±0.023
(b) Castrate partners	202	169	20	....	....	0.060 ±0.007	0.034 ±0.004	0.016 ±0.001
5 Parabiotic pairs— (a) Normal partners	245	247	21	0.963 ±0.067	0.304 ±0.028	0.222 ±0.051	0.142 ±0.035	0.045 ±0.010
(b) Castrate partners treated with rat testicular mush	272	209	21	....	....	0.060 ±0.005	0.039 ±0.004	0.018 ±0.003
8 Parabiotic pairs— (a) Normal partners	144	120	..	0.783 ±0.120	0.232 ±0.040	0.093 ±0.036	0.069 ±0.023	0.037 ±0.006
(b) Castrate partners treated with inhibin	151	131	19	....	....	0.049 ±0.010	0.020 ±0.002	0.013 ±0.002
5 Parabiotic pairs— (a) Normal partners	239	189	..	0.957 ±0.181	0.286 ±0.034	0.071 ±0.010	0.073 ±0.015	0.027 ±0.005
(b) Castrate partners treated with androtin	227	177	20	....	....	0.221 ±0.058	0.182 ±0.016	0.064 ±0.015

of androtin, because of the marked decrease in the size of the prostate in the castrated animals receiving the injections. Therefore it seemed possible, despite the similarity of the results with androtin, that some active principle other than androtin might be present in testes.

The results secured in normal animals not in parabiosis have furnished more convincing evidence of the duality of the testicular secretion. If our theory is tenable, the injection of a testicular substance containing inhibin should cause pituitary hypofunction in normal animals. This would result, of course, in hypofunction of the testes and hence atrophy of the prostate and seminal vesicles. A number of normal rats have received injections of the expressate from whole beef testes with the result that there was a marked diminution in the size of the secondary sex organs in all the rats after three weeks' treatment. In each case the organs were examined grossly by opening the abdomen before the experiment was commenced. A control group of animals showed that this preliminary observation did not result in any change in their size.

The results obtained by the injection of this material are in marked contrast to those obtained when androtin was injected into normal animals. In the latter group there was a definite increase in the size of the secondary sex glands. The results of this series of experiments led us to believe that the substance in testes which causes prostatic atrophy cannot be androtin, even though androtin and the testicular extract containing inhibin do produce similar effects in parabiotic animals.

To elicit further proof of this fact, the testicular expressate was extracted with ether to remove the fat-soluble hormone, androtin. The insoluble portion was injected into normal rats, and prostatic atrophy was produced. The results of all these experiments on normal rats are shown in Table 2. The findings seem to indicate that some substance in the testes other than androtin must cause the atrophy of the secondary sex glands.

TABLE II

EFFECTS OF INJECTION OF VARIOUS TESTICULAR EXTRACTS IN PARABIOTIC RATS

	Original Average Weight	Final Weight	Days	Mean Ratios of Organ Weight to Body Weight and Standard Deviations of the Means				
				Testes	Epididymis	Seminal Vesicles	Ventral Prostate	Dorsal Prostate
16 Normal controls	gm.	gm. 191	..	1.131 ±0.048	0.337 ±0.015	0.248 ±0.026	0.150 ±0.012	0.054 ±0.006
6 Castrate controls	207	200	22	....	....	0.043 ±0.002	0.015 ±0.001	0.010 ±0.000
9 Normal animals injected with inhibin	157	144	20	1.079 ±0.080	0.337 ±0.028	0.089 ±0.012	0.096 ±0.013	0.031 ±0.003
7 Normal animals injected with androtin	223	223	20	0.986 ±0.107	0.354 ±0.035	0.364 ±0.086	0.256 ±0.033	0.076 ±0.016
3 Normal animals injected with ether insoluble testis extract	205	165	18	1.268 ±0.076	0.347 ±0.027	0.079 ±0.022	0.099 ±0.028	0.030 ±0.010

Some objections have been raised to the theory of dual endocrine function of the testes on the ground that the testes are very sensitive to toxic substances and to changes in temperature and that hence it is possible that a great many substances might be capable of depressing their activity and thus causing prostatic atrophy. Indeed Bucheim (4), on the basis of unconfirmed experiments, has asserted that the injection of small doses of fuel oil into rats will cause prostatic atrophy. This possibility naturally demands caution in the interpretation of all results, but we have been able to show in our experiments that rats may receive extremely toxic preparations without suffering prostatic atrophy. We have prepared muscle extracts according to the same process used in the preparations of testicular extracts containing inhibin, and have injected them into rats without producing any change in the size of the prostate. No prostatic atrophy occurred following the daily injection of a typhoid vaccine containing 3,000,000 organisms. The injection of peptone resulted in some atrophy in one animal, and some enlargement of the secondary sex glands in another. On several occasions, when administering various fractions of the testicular preparation, some of the preparations which contained no inhibin were so toxic that many of the animals died, yet no prostatic atrophy was observed in the surviving animals that received the same dose. These results certainly argue against the possibility that the prostatic atrophy is caused by the injection of non-specific toxic substances.

Some preliminary investigations on a small series of animals may also be mentioned. The injection or feeding of the testicular substance containing inhibin has caused cessation of the estrus cycle in female rats, and the feeding of whole testes by mouth to guinea pigs has greatly decreased the secretion of seminal fluid as shown by ejaculation tests.

In addition to these experiments in our own laboratory, one or two other investigations might be mentioned which seem to add weight to the theory that the testes secrete a substance which inhibits pituitary activity. Evans (5) has shown by implantation experiments that the pituitary glands of cryptorchid animals contain more gonadotropic hormone than do the hypophyses of normal animals. Therefore, in cryptorchidism the testes lose their ability to depress pituitary activity, but do not lose their ability to maintain the prostate, for there is no sign whatsoever of atrophy in the prostates of cryptorchid rats. We interpret this as indicative of the dual endocrine nature and function of the testes.

The work of Myers and his collaborators (6) is also confirmatory. They mention that Moore and Gallagher demonstrated prostatic hypertrophy by the injection of testicular extracts. They repeated the experiments by feeding whole testes, in place of injecting extracts, and obtained very definite atrophy of the prostate and seminal vesicles of the animals thus treated. They offer no explanation for the results, but it appears to us that they furnish striking evidence in favor of the existence of the testicular chalone, inhibin, for it is well known that the fat-soluble hormone, androtin, is almost wholly ineffective when administered orally.

SUMMARY

1. When two adult male rats are surgically united by celeoanastomosis so that they live in parabiosis and one is castrated, the sex glands of the normal partner are stimulated while the secondary sex glands of the castrated animal become atrophic.

2. Injection of the comb-growth producing hormone, androtin, into the castrated partner prevents the stimulation of the sex glands in the normal partner and prevents secondary sex gland atrophy in the castrate.

3. A second substance in the testes, called inhibin, when injected into the castrate, also prevents the changes normally observed.

4. Androtin causes hypertrophy of the secondary sex glands of normal male rats.

5. Inhibin causes atrophy of the secondary sex glands of normal male rats, a decrease in the amount of material ejaculated by guinea pigs and a cessation of the estrus cycle in female rats.

6. Nonspecific toxic substances do not produce the same effect as inhibin.

We wish to thank Dr. William E. Lower for his cooperation and for his suggesting that we investigate prostatic hypertrophy.

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QUANTITATIVE STUDIES ON THE REACTION OF THE ANTERIOR PITUITARIES OF IMMATURE FEMALE RATS TO EXTRACTS OF PREGNANCY URINE*

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It has been found by Severinghaus (1934) and Wolfe (1934) that injections of extracts of pregnancy urine induce marked changes in the anterior pituitaries of immature female rats. The latter reported that such injections induced a marked increase in the weight of the ovaries of immature female rats and a variable increase in the weight of the pituitaries. The ovaries contained many corpora lutea, partially luteinized follicles and unluteinized follicles of all sizes. In the anterior pituitaries the most prominent morphologic changes were in the basophiles, which were usually enlarged and exhibited a marked loss of granules. Less outstanding was the morphologic evidence of loss of granules from the eosinophiles; this loss in most instances involved only a minority of the cells. The relative level of the eosinophiles was usually decreased, while that of the chromophobes was increased. Of equal interest was the fact that these extracts were without effect on the anterior pituitaries of castrated female rats.

In the studies above referred to, in which extracts of pregnancy urine were used, the immature rats received injections of 25 to 75 rat units daily for periods of 7 to 15 days. Daily injections of 25 units were as effective in obtaining the ovarian and anterior pituitary reactions mentioned above as were 75 units. However, rats which received injections for the longer periods of time usually, but not invariably, exhibited a greater weight increase of the ovaries and pituitaries and exhibited more marked morphologic changes in the anterior pituitaries. Since the morphologic reaction of the anterior pituitary to the anterior pituitary-like factor is of primary importance in the study of certain phases of the reproductive cycle, we have thought it important to study more exactly the effect of varying the length of the injection periods and the amount of the extract injected. With this point in mind, the following series of experiments were set up.

Complete litters of female rats, 21 days of age, were used. Two rats from each litter received *daily* injections of 25 units of pregnancy urine extract.† One of these two was sacrificed on the sixth day of the experiment, 120 hours after the first injection or 24 hours after the last of five injections. The other animal of the two was sacrificed on the eleventh day

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†Pregnancy urine extract, Follutein, was furnished by E. R. Squibb through the courtesy of Dr. J. J. Durrett.

of the experiment, 240 hours after the first injection or 24 hours after the last of ten injections. Two littermate sisters, from the same litter as above, received a *single* injection of 25 units of the extract. One of these was sacrificed on the sixth day after this single injection and the other on the 11th day. A littermate control was sacrificed when the two rats which had been on experiment for five days were killed. There were no controls for the animals on experiment for ten days. In other words, we have tested the effect of both daily and single injections running for two definite periods of time; i.e., 5 and 10 days.

Vaginal smears were made daily throughout the experimental period (after the vaginae had opened, usually on the 5th day). At autopsy, body, ovary and pituitary weights were recorded. Serial sections of the ovaries and representative sections of the vaginae and uteri (stained in hematoxylin and eosin) were studied. The pituitaries were prepared for study by the standard method previously described (Cleveland and Wolfe, 1932). Five representative sections, selected from complete serial sections, were studied and differential cell counts were made.

EXPERIMENTAL RESULTS

Pituitary and ovary findings on controls. Reference to Table 1 shows that the mean weight of the ovaries of the control rats, killed at the same time as the experimental rats which were killed on the sixth day, was 15.5 mg. Histologic examination revealed that, with two exceptions, the ovaries, as well as the vaginae and uteri, were infantile. The mean weight of the pituitaries was 2.2 mg. Morphologic examination revealed that the percentages of the eosinophiles ranged from 33.7 to 40.4 per cent; the mean percentage was 37.1.‡ These cells were well filled with granules which stained intensely with orange G. The basophiles were abundant and with two exceptions were well filled with granules which stained deeply with aniline blue. The mean level of basophiles which were completely filled with granules was 7.2 per cent, while the mean level of those which exhibited granular loss was only 1.9 per cent. The pituitaries of this group of controls were in every way similar to those of a standard group of 53 normal immature rats on which data have recently been published (Wolfe, 1935). The two exceptions noted above were in the anterior pituitaries of two rats which exhibited a precocious sexual maturity. The vagina of each of these rats was cornified and the uterus distended. The basophiles of these two animals exhibited granular loss; a morphologic finding similar to that found in mature females in oestrus.

Pituitary and ovary findings in experimental groups which received a single injection and were killed on the sixth and eleventh days of the experiment, respectively. The ovaries of the rats which received a single injection of pregnancy urine extract and were sacrificed on the sixth day after the injection were only slightly increased in weight when compared

‡Means of percentages and standard deviations of the various cell types are omitted for purpose of conserving space.

with those of the controls; usually only 3 or 4 mg. (see Table 1). The mean ovary weight of this group was 18.6 mg. Histologic examination revealed that these ovaries in some instances contained 2 to 4 mature follicles, which in many instances were partially luteinized. In a majority of the animals, however, the ovaries contained 1 to 4 small corpora lutea. It was not ascertained with certainty whether all such corpora resulted from direct luteinization or followed ovulation. However, in many an imprisoned ovum was found. The vaginae of this group of rats were cornified in every instance and the uteri presented a high pseudostratified epithelium.

The pituitaries of this group of rats were in some instances slightly increased in weight when compared with those of the controls. This finding was inconstant, however, and reference to Table 1 will show that the mean weight of these glands was only slightly greater than that of the controls. Morphologically, the pituitaries of these animals exhibited loss of granules from the basophiles (Table 1). There were no definite mor-

Class Intervals in Percentage	Killed on the Sixth Day			Killed on the Eleventh Day	
	Control	Single Injection	5 Daily Injections	Single Injection	10 Daily Injections
Eosinophiles—					
20.0–24.9	..	..	4	..	1
25.0–29.9	..	1	9	..	10
30.0–34.9	..	8	6	6	4
35.0–39.9	12	8	..	6	..
40.0–44.9	3	2	..	4	..
Basophiles—Granular—					
0.0– 1.9	..	2	4	11	14
2.0– 3.9	2	14	11	5	1
4.0– 5.9	1	2	4	..	..
6.0– 7.9	7	1	..	..	..
8.0– 9.9	5	..	..	..	..
Basophiles—Non-granular—					
0.0– 1.9	8	..	..	..	1
2.0– 3.9	5	2	5	3	5
4.0– 5.9	2	14	6	7	6
6.0– 7.9	..	3	6	6	3
8.0– 9.9	..	..	2	..	..
Chromophobes—					
45.0–49.9	2	2	..	..	..
50.0–54.9	11	4	..	5	..
55.0–59.9	2	10	1	10	..
60.0–64.9	..	2	11	1	5
65.0–69.9	..	1	7	..	10
Number rats per group	15	19	18	16	15
Mean rat weight	51.2 gm.	54.5 gm.	56 gm.	70.2 gm.	74.0 gm.
Mean ovary weight	15.5 mg.	18.6 mg.	51 mg.	18.5 mg.	73.5 mg.
Mean pituitary weight	2.2 mg.	2.6 mg.	3.2 mg.	3.0 mg.	4.3 mg.

TABLE 1.—Frequency distribution table, giving detailed quantitative data. Class intervals given in percentage. Total rats in each group, and mean rat, ovary and pituitary weights are given.

phologic changes in the eosinophiles. Reference to Table 1 will show that the level of these cells was practically the same as that found in the controls. Briefly, the only changes induced in the anterior pituitaries of immature female rats killed on the sixth day after a single injection of 25 units of pregnancy urine extract was a loss of granules from the basophiles. The pituitaries of this group of rats were in every way similar to those of two control rats which had come into oestrus spontaneously.

The ovaries of the rats which received a single injection of 25 units of pregnancy urine extract and were killed on the 11th day of the experiment weighed practically the same as those of animals which received the same dosage but were killed on the 6th day of the experiment. Practically all these ovaries contained 1 to 5 corpora lutea and usually no large follicles. With one exception the vaginae of these rats were regressive, the vaginal wall being relatively low and infiltrated by many leucocytes. It is important to remember that these animals were in a definite oestrus on the fifth day of the experiment, but that they returned to dioestrus by the seventh or eighth day. One rat of this group failed to show this condition; the vagina of this animal was definitely mucified and the uterus considerably enlarged. The pituitaries of this group of rats were only slightly increased in weight over those found in immature control rats. However, definite morphologic changes were found. The basophiles exhibited a rather marked loss of granules, which in most instances was more prominent than that found in the rats which had received a single injection and were killed on the sixth day of the experiment. As the result of this granular loss, the percentage of non-granular basophiles was increased (Table 1.) There were no changes in the eosinophiles; reference to Table 1 will show that in every instance the relative level of these cells was within the normal limits. There was no morphologic evidence of granular loss from these cells except in one animal, which exhibited a mucified vagina (noted above).

Pituitary and ovary findings in experimental groups which received daily injections and which were killed on the sixth and eleventh days of experiment, respectively. The ovaries of the rats which received daily injections and were killed on the sixth day of the experiment were markedly increased in weight; the mean weight was 50.5 mg. They contained many corpora lutea, which were much larger than those found in rats receiving single injections. There were also many follicles, some of which were partially luteinized. The vaginae were cornified and the uteri presented a high pseudo-stratified epithelium. The pituitaries were significantly and constantly increased in weight; the mean weight was 3.4 mg., while the mean weight of the pituitaries of the controls was only 2.2 mg. Morphologic examination revealed that the basophiles had lost granular material. The percentage of the basophiles containing granules was reduced to a mean level of 3.6, while that of the non-granular forms had increased to 5.1 per cent. It is significant that the granular loss from the basophiles of animals receiving 5 *daily* injections of 25 units of pregnancy urine extract was no greater than that of the basophiles of rats which received a *single* injection of 25 units. The eosinophiles in this group were significantly reduced in percentage. In 13 of the 16 animals in this group the level of these cells was below 30 per cent; the mean level was 27.5 per cent. The mean level in the controls was 37.9 per cent. (See Table 1 for frequency distribution.) Many of the eosinophiles in this group of pitu-

itaries exhibited rather slight but definite morphologic evidence of loss of granules.

The ovaries and the pituitaries of rats which received daily injections and were killed on the eleventh day of experiment were further increased in weight when compared with those of the rats killed on the sixth day. The ovaries were stimulated to a mean weight of 73.4 milligrams and contained many large corpora and follicles, some of which were partially luteinized. The vaginae of these rats were usually mucified but in some instances were cornified. The pituitaries of this group of rats had a mean weight of 4 mg., which is a definite increase over the weight of the pituitaries of immature control rats of the age and weight (Wolfe, 1935). The anterior pituitaries of this group exhibited an extreme loss of granules from the basophiles. The mean level of the basophiles filled with granules was only 1.7 per cent, while the mean level of the non-granular basophiles was 5.1 per cent. The mean relative percentage of the eosinophiles was significantly reduced and many cells showed a definite loss of granules. Such cells often presented a greatly enlarged negative image of the Golgi apparatus. The mean level of these cells was 28.1 per cent, while in controls of this age the mean level of these cells was 37.1 per cent (Wolfe, 1935).

The chromophobes in these two groups were considerably increased in relative percentage (Table 1). Many were very large and had scant cytoplasm, which was light blue or nearly colorless. Others were small and had dense blue cytoplasm. In both, the negative image of the Golgi apparatus was often greatly enlarged.

Observations on mitotic activity. Three sections from each gland were carefully examined for the presence of mitoses. In the controls we found an average of 6.4 and 1.8 mitoses per section in the chromophobes and the eosinophiles, respectively. In the two experimental groups killed on the sixth day of experiment (both those receiving single and daily injections) an increased number was found, which was practically the same in both instances. Considered together, there was an average of 11.4 and 5.8 mitoses per section in the chromophobes and the eosinophiles, respectively. In the rats receiving a single injection and which were killed on the eleventh day of experiment this average had dropped to 7.6 and 4.5 mitoses per section in the chromophobes and the eosinophiles, respectively, while in the rats which had received daily injections throughout this period the rate of mitotic division was still high; an average of 10.9 per section was found in the chromophobes and 4.4 per section in the eosinophiles. Our data would indicate that injections of pregnancy urine extracts definitely increased the rate of mitotic division and that in the five-day experiments the single injections were as effective as the daily. These figures are averages and there was considerable variation in the various glands. However, they give an accurate index of the rate of division in the various groups.

DISCUSSION

These studies demonstrate that the anterior pituitaries of immature female rats not only react in a characteristic and definite fashion to injections of the anterior pituitary-like factor of pregnancy urine, but also show that the nature of this reaction is, to a degree at least, dependent on the period of injection and the amount of the substance injected. It is also demonstrated that the intensity of the anterior pituitary response is closely correlated with the response of the ovaries. Thus in rats receiving *single* injections and killed on the sixth and eleventh days after the injection the ovaries exhibited a few mature follicles and corpora lutea. However, it seems doubtful that these corpora became active, since the vaginae of the rats killed on the eleventh day after injection were lined by a low epithelium, infiltrated by leucocytes. Correlated with this finding in the reproductive organs, a rather constant picture was presented by the anterior lobe of the pituitary, notably, a loss of granules from the basophilic cells. This reaction is similar to that found in the anterior pituitaries of rats killed during normal oestrus.

In the rats receiving daily injections were found many large corpora lutea which were evidently active. The vaginae of the rats killed on the eleventh day were, in most instances, mucified and similar to those of normal female rats killed from the ninth to the thirteenth days of pseudopregnancy. The anterior pituitaries of such rats not only exhibited marked degranulation of the basophiles but also exhibited a moderate loss of granules from the eosinophiles. Our findings would indicate, therefore, that loss of granules from the eosinophiles and a reduction in their relative percentage was associated with the presence of active corpora lutea in the ovaries, an observation previously reported by us.

SUMMARY

A group of immature (21 days) female rats received a *single* injection of 25 units of pregnancy urine extract. Approximately half of these rats were killed on the sixth day following the injection, the remaining half on the eleventh day. Another group received *daily* injections and were killed at the same time intervals as those above. Littermate sisters were used in these and a control group. Rats receiving a *single* injection and killed five days later presented ovaries with a few mature follicles or two to five small corpora; the vaginae were cornified. The pituitaries were slightly increased in weight and morphologically showed degranulation of the basophiles. Those receiving a *single* injection and killed ten days later presented ovaries with two to five small corpora and regressive vaginae. The basophiles of the anterior lobe were markedly degranulated. Rats receiving five *daily* injections presented ovaries increased in weight (average, 50.2 mg.) and containing many large corpora and follicles; the vaginae were cornified. The pituitaries were increased in weight and presented definite granulation of the basophiles and slight granular loss from the

eosinophiles, which were reduced in percentage. Rats receiving ten *daily* injections presented ovaries markedly increased in weight (average, 70.2 mg.) which contained many corpora and follicles; the vaginae of most were mucified. The pituitaries were markedly increased in weight and showed degranulation of the basophiles and moderate granular loss from the eosinophiles which were also reduced in percentage.

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Book Reviews

YEARBOOK OF NEUROLOGY, PSYCHIATRY AND ENDOCRINOLOGY. Hans H. Reese, Harry A. Paskind and Elmer L. Sevringhaus. Pp. 782. Year Book Publishing Company, Chicago, Illinois. 1934.

This well-known yearbook formerly edited by Dr. Peter Bassoe—more recently with the assistance of Dr. F. G. Ebaugh—appears this year under new editorship. Dr. Sevringhaus assumes responsibility for Endocrinology.

The section on this subject (252 pages) opens with an introduction which presents a judicious epitome of the established knowledge of the glands. A selection of articles that substantially add to knowledge of each of the glands is then presented. This constitutes, however, no mere collection of conventional abstracts; by the use of introductory notes and appended critical comments the mass of material is placed in an intelligible frame of reference. The result is an organized production that serves admirably to place the reader in contact with the more important lines of advancement in this field. The editor throughout displays a masterly grasp of the subject and skill in pointing practical relationships. The work will be welcome and useful.

CLINICAL PATHOLOGY OF THE JAWS. Kurt H. Thoma, Charles C. Thomas, Springfield, Ill., and Baltimore, Md. 1934.

"This volume represents the results of investigation carried on in the Laboratory of Oral Pathology of the Harvard University Dental School and the systematic study of cases collected in private practice of oral surgery for a period of almost twenty years."

While it is of interest chiefly to the dental profession the book will be significant to endocrinologists in that it brings together a considerable amount of material from the literature on the relation of the various glands to dental health and disorders. This material is supplemented by a number of interesting first-hand studies of dental conditions in various of the endocrinopathies.

The book is excellently printed and copiously illustrated. The wealth of new material reported will demand the serious attention of all serious students of the field of dentistry.

Abstract Department

The adrenals and anaesthetic hyperglycemia. Banerji, H. and C. Reid, J. Physiol. **78:** 370. 1933.

Three methods, operative, indirect and comparative, were used to determine the rôle of the adrenal glands in the glycemic response to open etherization. In the operative method studies were made on 30 rabbits; after double adrenalectomy (10), unilateral adrenalectomy with contra-lateral medulli-adrenalectomy (10), and double medulliadrenalectomy or double denervation and decapsulation (10). The glycemic response during surgical etherization for 30 minutes was found to be less after these operations than in the intact animal. In the indirect method combinations of anesthetics and narcotics were used. The glycemic responses of normal and amytalized animals to ether, to morphine narcosis, and to adrenaline were determined. Dogs as well as rabbits were used in these experiments. It was found that amytal inhibited the usual hyperglycemia occurring in ether or chloroform anesthesia and in morphine narcosis, but not that following subcutaneous injection of adrenaline. For the comparative method, anesthetic glycemic response was determined in two groups of rabbits, one which had shown previously a large increase in blood sugar in response to injected adrenaline and the other less sensitive to adrenaline. The response was greater in the more sensitive group. It was concluded that the adrenals are concerned partly in increasing the blood sugar during 30-minute surgical anesthesia with ether or chloroform.—L. C. W.

Addison's disease due to malignant involvement of the solar plexus. Bicknell, F., Brit. M. J. **2:** 206. 1934.

A few cases have been reported in which Addison's disease existed in patients with apparently healthy adrenal glands. No recent instances apparently, however, have been seen. The case is reported of a man of 50 who three years previously began to have epigastric pain. When seen by the author he appeared to be more sunburned than usual and the abdominal pains had become severe. The pigmentation increased until the skin of the nipples and genitalia became a deep black. The greying hair and mustache became much darker. There were sudden increases in the pigmentation—always associated with severe abdominal pain. He became progressively weaker, ultimately having to stay in bed. He entirely lost the sense of taste and smell. The blood pressure gradually fell from 144/98 to reach 96/57 mm. Hg. two weeks before death. A rather marked anemia was present. The blood urea was 32 mg. per 100 cc. The fasting blood sugar was normal. He became gradually weaker and vomited incessantly and finally died. Autopsy showed marked emaciation, extreme pigmentation of a brownish type, mainly of exposed parts. The adrenals appeared to be normal. A large mass of carcinomatous gland pressed directly on the coeliac plexus.—R. G. H.

Addison's disease; report of case treated by eschatin. Cheney, W. F., California & West. Med. **39:** 262. 1933.

This is the report of a case of Addison's disease occurring in a woman of 27 who is still apparently well more than two years after eschatin treatment was instituted. The patient still takes two cc. of eschatin by injection daily.
—I. B.

The effect of insulin and epinephrine on the amino acid content of the blood of adrenalectomized rabbits. Davis, B. L., Jr., and W. Van Winkle, Jr., J. Biol. Chem. **104:** 207. 1934.

The hypothesis that insulin injected into adrenalectomized rabbits caused a hypoaminoacidemia was questioned, since epinephrine is also known to cause this phenomena. Twelve adrenalectomized rabbits weighing from 2 to 3 kilo were injected with from one-fourth to 1 unit of insulin per kilo. To prevent the blood sugar being reduced to the convulsive level, 10% glucose solution was given. Samples of blood taken at 1, 2, 3, 5, and 8 hours, respectively, showed no lowering in amino acid content, as was produced by administration

of insulin to normal animals. In similar experiments using epinephrine (0.1 to 0.2 mg. per kilo), a marked drop in amino acid nitrogen was noted. Thus the authors were able to prove that epinephrine rather than insulin was the responsible agent. Inorganic phosphorous showed a drop in the case of both insulin and epinephrine. Sugar was also determined. It was mentioned that the ability of insulin, acting indirectly through the adrenals, to cause hypoaminoacidemia, might be a possible means of testing for the adequacy of the medullary function.—Hazel M. Childs.

On the presence of "novadrenine" in suprarenal extracts. v. Euler, U. S., J. Physiol. **78:** 462. 1933.

Szent-Györgyi and his co-workers had recently found that the physiological activity of certain extracts of the adrenal medulla was greater than the adrenaline content as determined colorimetrically justified. They had suggested, therefore, that these extracts contained a more active form of adrenaline ("novadrenine"). Extracts from the bovine suprarenal medulla were prepared according to the method of Szent-Györgyi and his co-workers. The physiological activity of the extracts was determined by the blood pressure method in cats under ether and in rabbits anesthetized with urethane. Colorimetrical determinations were carried out by a spectrophotometric method based on the Vulpian (1856) reaction (Euler, 1933). This method was found to give figures in fairly close agreement with those found biologically. It was concluded that this agreement does not admit the assumption of a special, more highly active adrenaline-like substance. It was suggested that the colorimetric method used previously by Szent-Györgyi was less suitable for the determination of adrenaline, since the color is liable to fade away before the determination has been completed, thus giving unreliable results. In a footnote to this paper Szent-Györgyi accepts the views of v. Euler, and admits that the supposition of the existence of "novadrenine" has become superfluous.—L. C. W.

Studies on the adrenal; the oral administration of the cortical hormone and the use of fresh glands therapeutically. Grollman, A. and W. Firor, Bull. Johns Hopkins Hosp. **54:** 216. 1934.

The activity of the adrenal cortical hormone when administered orally was demonstrated. The hormone is non-protein in nature and stable in acid solution and is not affected by the stomach. It is inactivated in alkaline medium which probably accounts for the fact that five times the parenteral dose was required to elicit an equally beneficent effect when administered orally. Fresh or desiccated adrenal tissue proved to be highly toxic unless the medulla was completely removed immediately after removal of the glands from the body. Such glands are ineffective therapeutically because of the rapid destruction of the hormone by oxidative enzymatic processes. Cortical tissue rapidly heated to 100° C. preserves its hormonal content to a sufficient degree to offer a source of the hormone, as was demonstrated by feeding experiments in adrenalectomized dogs. A more practical means for oral therapy is desirable.—A. Grollman.

Accessory adrenals in white rats (Las suprarrenales accesorias de la rata blanca). Lascano-González, J. M., Rev. Soc. argent de biol. **10:** 28. 1934.

In white rats small nodules of cortico-adrenal tissue are always found which hypertrophy after adrenalectomy. The cells are of two types corresponding to the fascicular and glomerular layers of the adult adrenal; the former predominate. Sometimes the hypertrophied nodules come into contact with sympathetic ganglia and may be faced with these, in which cells resembling pheochromoblasts appear.—J. T. L.

Effect of adrenaline on circulating blood volume (La acción de la adrenalina sobre el volumen de la sangre circulante). Levin, E., Rev. Soc. argent. de biol. **10:** 456. 1934.

Circulating blood volume was determined by means of the vital red method, before and 8 to 20 minutes after subcutaneous injection of 1 mg. adrenaline in 1 cc., in 16 subjects. Corpuscle volume increased in all cases (1 to 12.5%, average 3.9%). In 8 subjects the blood volume decreased. It

increased in 6 and no change was observed in 2. Plasma volume decreased in 13 subjects. These results are very similar to those seen in moderate exercise. The author supposes that short-circuiting may occur in the pulmonary circulation, in which case part of the blood will circulate rapidly and the dye being unequally distributed an apparent decrease in blood volume will be registered. If there is no short-circuiting the dye will be evenly distributed and a real increase in blood volume will be registered.—J. T. L.

Reaction of eye to sympathicomimetic drugs after section of the pre- and post-ganglionary sympathetic fibres (La sensibilidad del ojo a diversas substancias simpático-miméticas después de seccionar las neuronas pre y post-ganglionares del simpático). Lewis, J. T. and F. P. Ludueña, Rev. Soc. argent. de biol. **10**: 447. 1934.

Approximately one month after sectioning the preganglionic fibres of the superior cervical ganglion, the eye on the denervated side responds more markedly than the other eye to intravenous injection of adrenaline, cocain and ephedrin. After subsequent extirpation of the S.C.G. hypersensitiveness to adrenaline persists, but the response to ephedrin gradually diminishes and becomes considerably smaller than that of the eye with intact innervation. In some cases opacity of the cornea was seen after section of the preganglionar fibre, which in one case disappeared after extirpation of the S.C.G.—J. T. L.

Influence of different diets on the nucleo-plasma relation of the cells of the suprarenal gland of the rat (Messungen an den Nebennierenzellen der Ratte. Einfluss verschiedener Ernährung auf die Kernplasmarelation). Kolliner, Martha, Ztschr. f. Anat. u. Entwcklingsgesch. **99**: 304. 1932.

Forty-six rats, weighing from 88 to 145 grams were fed for 7 weeks on various diets such as wheat, rice, milk and various mixtures of these with casein, salt mixtures, lard, butter, greens, yeast, cod liver oil, "oryzan" and meat. Of the animals, 16 lost weight; the rest gained. The ratio of the cross-section area of the entire cell to the cross-section area of the nucleus was determined for cells of zona glomerulosa, zona fasciculata, zona reticularis and of the medulla. In all regions except glomerulosa a diet of wheat casein and butter showed the greatest ratio (relatively large cell for the size of the nucleus). In zona glomerulosa, a diet of wheat, meat and greens produced the largest ratio. Rice alone, on the other hand, produced the smallest ratio (relatively large nucleus) in all regions.—A. T. R.

The significance of salt in the treatment of Addison's disease. Loeb, R. F. and D. W. Atchley, M. Clin. North America, **17**: 1317. 1934.

Because of the striking similarity of the state of acute adrenal insufficiency to a state of shock in diseases characterized by a loss of salt and water as in diabetic acidosis, intestinal obstruction and cholera, the authors undertook chemical studies in patients suffering from Addison's disease to determine whether the blood and interstitial fluids were depleted of basic salts. A case history is detailed in which a patient in extremis from sodium depletion was given a single dose of Eschatin intravenously, 9.5 gm. of NaCl per day by proctoclysis, 5 gm. per day by mouth in capsule form, and broth containing much salt. Striking improvement was noted within 4 days. Twenty-one days after admission she was discharged on a regime of 5 to 7 gm. of NaCl per day by mouth in addition to salt in the diet. Five months later she was placed on a salt-free diet but a week after this she returned to the hospital with a return of symptoms. Salt was again administered in heavy doses. No adrenal cortex extract was given. Improvement was again striking and the patient was discharged 8 days later feeling very well. Three other cases showed similar results. There does not appear to be any close relationship between the blood pressure level and the sense of well being, but the latter parallels to an amazing degree the concentration of sodium in the blood. It was observed that patients require 10 to 15 gm. of NaCl daily in addition to that contained in the diet. Experimenting on dogs, the authors performed adrenalectomy and confirm their theory that the salt is excreted and not merely transferred to the tissues. Despite the sense of well being, patients retain most of their pigmentation, are sensitive to cold and fatigue, and systolic blood pressure and sugar concentration in the blood tend to be low.—I. B.

Disappearance of vitamin C from the adrenals of scorbutic guinea pigs. Siehrs, A. E. and C. O. Miller, J. Nutrition, **8**: 221. 1934.

The silver nitrate staining reaction shows that there is a progressive decrease of ascorbic acid in the cortex of adrenals of guinea pigs having scurvy. The decrease is apparent on the second day of the scorbutic diet period. Minimum staining is obtained on the sixth day. The feeding of 3 cc. of orange juice to scorbutic guinea pigs is followed by the prompt reappearance of ascorbic acid in the cortex of the adrenals. An iodimetric method for the determination of the iodine reducing substances in the adrenals, which includes ascorbic acid, shows that there is a progressive decrease of these substances during the onset of scurvy.—Authors' Abst.

Spontaneous hypoglycemias in endocrinopathies. Blumgarten, A. S., M. Clin. North America, **17**: 79. 1933.

Spontaneous hypoglycemia is a condition frequently associated with various endocrinopathies. Dyspituitarism, adrenal insufficiency, and frequently hyperthyroidism may act as factors in the production of spontaneous hypoglycemia. Three cases of spontaneous hypoglycemia are presented: (1) A case with dyspituitarism developing from a low carbohydrate diet and the development of the symptomatology from the administration of thyroid; (2) a case resulting from insufficient adrenal secretion thereby eliminating the check on insulin secretion; and (3) a case developing as a premenstrual symptom in a constitutional asthenic with probable evidence of adrenal insufficiency.—I. B.

The nature and types of gynecomastia (Über Wesen und Formen der Gynäkomastie). Bredt, H., Ztschr. f. Konstitutionslehre, **17**: 29. 1932.

The different conceptions of the etiology (such as testicular trauma, tumors of the hypophysis, adrenals and testes, cirrhosis of liver, constitutional and familial factors) of enlarged mammary glands in men are discussed. An analysis of many cases from the literature and a few of his own leads the author to the conclusion that gynecomastia in man in a technical sense is a special type of hyperdevelopment in which the histogenesis is essentially normal and with no specific pathology. It is not an inflammatory enlargement. The condition is not necessarily indicative of intersexuality and only rarely is it due to sexual hormones. These cases can be grouped into those that are due to some hormone imbalance and those that represent defective constitution of the mammary gland itself, and a few that are best explained upon the combined operation of both of these factors. In three cases of gynecomastia with cirrhosis of the liver, the testicular damage which was present, was ruled out as a causal factor of the mammary hypertrophy. In other cases of a dysontogenetic character, the injured and underdeveloped testes were similarly ruled out as the cause of the gynecomastia.—A. T. R.

Inhibitory hormones and the principle of inverse response. Collip, J. B., Ann. Int. Med. **8**: 10. 1934.

A summing up and evaluation of two years of work. It was found that animals treated for several weeks with thyreotropic hormone became non-reactive to it. Blood serum of the non-reactive subjects injected into normal animals caused these also to become non-reactive to this hormone. The inhibitory principle was demonstrated in the blood of rats, rabbits, dogs, goats and horses after treatment of six weeks or longer with adequate amounts of the thyreotropic extract. The onset of a similar non-reactivity was observed in hypophysectomized rats treated for 40 days with purified growth hormone. The specificity of such anti-substances is indicated by the fact that rats treated with ovary-stimulating placental hormone for a long period and in which the ovaries have become non-responsive do respond to the maturity factor contained in anterior pituitary. Likewise, rats rendered non-responsive to the maturity factor respond to placental hormone. The serum of animals treated for weeks with placental hormone inhibits the effect of placental extract in the immature female animal. The serum of a horse treated for three months with the pituitary growth hormone was shown to antagonize the effect of the growth hormone on the hypophysectomized rat. Similarly, a substance inhibitory to the ketogenic effect of the anterior pituitary extract was induced in horses. It is suggested that the production of such inhibitory substances represents a normal protec-

tive mechanism to prevent the subject from being overinfluenced by an excess of autogenic hormone. The principle of inhibitory hormone can account for differences in responsivity of different species to a given hormone as well as minor variations within a species. It indicates that any supposedly hypohormone state may actually represent a hyperinhibitory hormone condition. The complex of hormone and inhibitory principle can serve as a buffer. When both components are circulating at a high titre the subject is relatively unresponsive to the administration of more of the hormone. This latter suggestion is designated "the principle of inverse response." These findings suggest that in the clinical use of gland material the physician should be on his guard against setting up an overproduction of the inhibitory principle as the result of too persistent treatment. The suggestion is also obvious that if the inhibitory hormones can be isolated they will afford a means of treating the condition of overactivity of glands.—R. G. H.

The chemical transmitter at synapses in a sympathetic ganglion. Feldberg, W. and J. H. Gaddum, J. Physiol. **81:** 305. 1934.

The superior cervical sympathetic ganglia of cats were perfused with eserinized Locke's or Ringer's solutions. The perfusates collected during stimulation of the cervical sympathetic were capable of lowering the blood pressure of cats, of contracting eserinized leech muscle, of depressing the inotropism of the frog's heart and the chronotropism of the rabbit's auricle. All these effects were abolished by atropine. These perfusates also caused contraction of the eserinized frog's rectus abdominis. A quantification of the potency of the solutions in terms of acetylcholine by all the methods mentioned yielded similar figures. The authors conclude that acetylcholine is liberated at the ganglion on stimulation of the preganglionic nerve fibers and that this acetylcholine is probably responsible for the activation of the post-ganglionic neurones.
—A. Rosenbleuth.

Trends in gynecology and obstetrics during 1933. Greenhill, J. P., Am. J. Obst. & Gynec. **28:** 307. 1934.

This article includes a useful summary of authoritative opinions and epitomes of results obtained in the clinical use of endocrine products in the field of women's diseases.—R. G. H.

Endocrine dyscrasias and mental disorders. Hutton, J. H., R. Brandon, C. F. Read and J. T. Nerancy, Illinois M. J. **64:** 242. 1933.

This is a preliminary report of procedures undertaken with the intention of determining whether there is a relationship between endocrine disturbances and mental disorders and whether correction of the former would assist in the treatment of the latter. This laudable work is still being pursued. Only young patients, less than age 45 (except a few cases in the menopause) and recently admitted to the clinic, are accepted. Patients are subjected to the usual psychiatric and general medical examination by the regular staff of the hospital. Without disturbing any psychiatric regime instituted and with all non-endocrine conditions eliminated so far as possible, those showing endocrine stigmata are sent to the endocrine clinic. Paretics, postencephalitics, arteriosclerotics and manic-depressives are, as above, excluded from this rule. The chief endocrine stigmata considered by the ward officers are: Statural excesses or deficiency; disproportion between upper and lower measurements; dystrophies of skin and appendages; unusual hair distribution or deficiency; peculiarities or excess or deficiency of pigmentation; obesity and its distribution; menstrual disorders. The chosen patient is subjected to a general endocrine examination by the extramural member. Special laboratory studies are ordered at this examination, if recent ones are not already part of the patient's record. The patient is seen at the next visit and a definite diagnosis made on the basis of the findings, considered in the light of the data accumulated previously, and a course of treatment is outlined. Check-ups are made at intervals of 2 to 4 months. The opinion of the doctor immediately in charge of the patient is solicited as to his or her condition. The authors conclude that there is no doubt, both from the data collected and from other reports on the subject, that endocrine therapy offers definite promise of the alleviation of some cases of some forms of mental disease. A small section of so-called insanity undoubtetdly will eventually be found due to endocrine dyscrasias amenable to endocrine therapy.—I. B.

On the presence of a diuretic hormone in the mucous membrane of the intestines (Sulla presenza di un ormone diuretico nella mucosa intestinale). Manzini, C., Biochem. e terap. sper. **21:** 185. 1934.

Intestinal extract, obtained by the technique of Ambard and Schmid, dissolved in 1 cc. of water was injected into the vena marginalis of a number of rabbits. The dosage varied between 0.05 and 2 mg. per kilogram of body weight. The urine was collected every 10 minutes directly from the ureters which had been previously operatively exteriorized. In all the experiments an abundant diuresis was obtained immediately after the injection of the extract. The greatest elimination was observed during the first 20 minutes after injection, after which the diuresis gradually approached normal values. Control experiments with extracts of liver, spleen, and kidney gave negative results. In the normals in which dosages of 10, 8, 6, and 4 mg., respectively, were injected, such extensive polyuria appeared that the animals died within two or three hours with the symptomatology of water inanition. In these four animals the determination of hemoglobin of the blood with Burcher's method revealed a marked hydremic state. As to the mechanism of action of the intestinal hormone, the author cannot yet form a final opinion.—A. Angyal.

Growth and basal metabolism; basal metabolism of pre-school children. Nakagawa, I., Am. J. Dis. Child. **47:** 963. 1934.

This is a report of the study of the basal metabolism of 31 pre-school Japanese children, 15 boys and 16 girls, between the ages of 3 years and 10 months and 6 years and 6 months. The basal heat production per 24 hours increases with age as do weight, height, sitting height, chest girth and other physical measurements. However, the basal heat production per unit of body weight and of surface area decreases with age as do the pulse rate and the respiratory rate. There is a difference between the heat production in boys and girls but the percentage of difference is not as great as between the two sexes in adult life. All the children showed a total heat production above the Benedict-Talbot standards and the percentage of deviation agrees with that noted for oriental children by Wang and Hawks in the United States.—M. B. G.

Obesity in children. Nixon, N. K., J. Pediat. **4:** 295. 1934.

The author feels that the obese child can be successfully treated by restriction of diet and increased muscular activity without the addition of endocrine extracts except thyroid which may be used occasionally. He reports the results obtained in children with and without organotherapy. He does not state the number of children under study and does not give any figures. Surgical pituitrin was given intramuscularly twice a week for a period of approximately two years in amounts necessary to produce abdominal cramps. Thyroid and antuitrin were given "when indicated." In addition the children were placed on a diet of approximately 850 calories made up of proteins, 60 grams; carbohydrates, 110 grams, and fats 19 grams. The majority lost weight and the results were satisfactory. For the past two years all cases of obesity were given the same diet with supervised exercise but without any pituitary extract. Desiccated thyroid was administered to the occasional child who apparently did not respond to this treatment. The results in the second series appear to be just as satisfactory as in the first series with organotherapy.—M. B. G.

Living grafts of endocrine glands. Stone, H. B., J. C. Owings and G. O. Gey, California and West. Med. **38:** 409; **39:** 10. 1933.

These two papers constitute a fairly thorough review of the subject with illustrative case histories. The success of grafts of endocrine glands depends on at least three principal factors, and possibly others not yet known. Those factors are the site at which the graft is placed, the physical form of the grafted tissue, and the chemical adjustment of the graft to the host. The graft should be placed in a location of loose structure free from pressure near a good blood supply but not too vascular, and easy of access. The graft should be either in finely divided fragments or in the form of tissue cultures grown from the original gland. Such forms of graft permit the individual cells to come in close and quick contact with nutrient substances without depending at first upon the development of an organized blood supply. The chemical adaptation of graft to host may be sought by growing the graft as a tissue culture, in a

medium made up from fluids of the host's body, thus providing an opportunity for preliminary adjustment of the graft to its future chemical environment. By the observation of these requirements in a series of experiments thus far not sufficient to be conclusive, a promising measure of success has been attained.
—I. B.

The internal secretion of the corpus luteum (Untersuchungen über die innere Sekretion des Corpus luteum). de Fremery, P. A., A. Luchs and M. Tausk, Pflügers Arch. f. d. ges. Physiol. **231:** 341. 1932.

The authors confirm the work of Knaus, Corner and Allen, Clauberg, and Hisaw and his co-workers on the physiological action of the corpus luteum hormones. In addition they state that they have been able to relax the ligamentous symphysis of virgin female guinea pigs by the injections of crystalline oestrin.—S. L. Leonard.

X-irradiation of the ovaries of guinea pigs and its effect on subsequent pregnancies. Genther, I. T., Am. J. Anat. **55:** 1. 1934.

Ovaries of sexually mature guinea pigs were x-rayed with a minimum sterilization dose or two-thirds of this amount. Oestrous cycles continued, though not always normally. In general, instances of failure to mate, infertile matings, and abortions in event of conception, increased with greater ovarian injury and subsequent more marked cyclic disturbances. The greater incidence of pregnancy occurred where cycles were only temporarily inhibited, then normal, and ovaries were functioning normally but had few follicles. No conceptions occurred where cycles were irregular with atypical indefinite oestrous openings, where ovaries contained almost no follicles but were still functioning normally. None occurred after the ovarian stroma was replaced by "interstitial gland," when cycles were very irregular with prolonged intense oestrous periods; a few occurred at the apparent normal cycles immediately following treatment, before the interstitial gland was established, but infertile matings and abortions were more numerous than successful pregnancies. Immature animals were unable to survive the full sterilization dose, thus no interstitial gland was produced. Smaller doses caused follicular destruction in varying degrees. At maturity, cycles were regular where ovaries were practically normal, irregular and atypical where ovaries had few follicles but were still functioning normally. A few pregnancies resulted, as in the above mature animals with similar histories. Histological findings are discussed.
—Authors' Abst.

Induced sterility. Guyer, M. F. and Pearl E. Claus, Physiol. Zoöl. **6:** 253. 1933.

The effects of sterility, induced in rats by anterior pituitary substance, testicular nucleoproteins, and spermatotoxins, on the oestrous cycle and on the ovary and the pituitary anterior lobe were investigated. Intraperitoneal injections of (1) anterior lobe substance led to follicular atresia; (2) of nucleoproteins, to retention of ova with frequent cleavage-like fragmentation of imprisoned ova, together with increase in the number of basophile cells in the pituitary anterior lobe; (3) of spermatozoa, to retention of ova because of what appears to be precocious luteinization, and to increase in anterior lobe oxyphiles. During all such treatments the oestrous cycle tends to persist, although it commonly becomes irregular, usually with periods of prolonged dioestrum. The infertility of rats rendered sterile by means of spermatotoxins could not be maintained by feeding dried testicular material. Spermatozoa become immobilized in the genital tracts of females in which spermatotoxins have been developed. Pituitaries of nucleoprotein-injected individuals were more potent than normal pituitaries in inducing precocious sexual development when transplanted into immature rats and mice.—Authors' Abst.

Excretion of prolan in the urine of old women (Uber die Ausscheidung von Prolan im Harn alter Frauen). Hamburger, C., Klin. Wchnschr. **12:** 934. 1933. Abst., Am. J. Obst. & Gynec. **28:** 625.

In a series of women well past the menopause the author found prolan in the urine in 75% of the cases. This corresponds almost exactly to the frequency with which prolan is found in the urine following castration, namely, 73%. The author also mentions the fact that he found prolan in the urine of 79% of castrated men.

Psychic feminization and hermaphrodization of males by means of female sex hormone (Progynon) (Psychische Feminierung und Hermaphrodisierung von Männchen durch weibliches Sexualhormon [Progynon]). Kun, H., Endokrinologie, **13:** 311. 1934.

Progynon benzoate in single doses of varying size was injected into castrated as well as non-castrated adult male rats. A period of from 2 to 11 months had elapsed between the time of castration and the experiments. Twenty-three castrated and 15 non-castrated animals were used. The effect in the castrated rats appeared regularly 48 hours after the injection, independent of the dose. The non-castrated animals showed the effect within 4 to 13 days. The dose of the single injection varied from 25 to 100,000 mouse units. The duration of the effect was not correlated with Progynon dosage. One animal with 50,000 mouse units showed the longest duration—26 days—of the specific feminine erotic reactions (lordosis of the spinal column, tremor of the ears, jumpy movements with sudden stops, squeaky voice). An injection of 100,000 mouse units caused lordosis for 10 days only in another animal. Of 2 animals with 20,000 mouse units, one showed the specific symptoms for 21 days and the other for 11. The threshold-dose was determined as 200 mouse units for non-castrated and 50 mouse units for castrated rats. In the injected, non-castrated males potency and libido were present at the same time as the specific feminine reactions. The hormone medication produced well-pronounced psychic hermaphrodites, who were able to react appropriately to a male or a female interchangeably. Only in cases of high doses of Progynon (starting with 10,000 mouse units) was there a distinct diminution of the male libido, progressing to complete impotence. In these cases the influence of the female hormone was so overwhelming that it obliterated the male libido completely and the animals acted as females.—G. L. Banay.

Observations on the results of castration. Lange, Berlin Correspondent, J. A. M. A. **102:** 1774. 1934.

Lange's observations are based mainly on a study of the official reports on soldiers wounded and operated on during the war. A follow-up of these patients supplies a more objective basis than the subjective accounts, which are often veiled or exaggerated. The results of later examinations are recorded in the army reports. In most cases, in addition to the final examination previous to discharge from the service, there was a second examination in 1919 and a further control examination during the period 1920-1922. Among 330 patients, the later life experiences of 250 were recorded. The age of the patient at the time of castration was below 25 in more than 100 instances. Some patients had undergone complete castration; some had been affected with bilateral tuberculosis of the testes, and still others presented small testicular remnants. Potency was seldom destroyed, and in some instances there seems to have been even an abnormal sexual desire, often with a discrepancy between potency and libido, or even inversion of the sex impulse. Of the patients undergoing complete castration, ninety married. During the first years after marriage there was no reduction of energy, working capacity or the like; in some instances even an increase in artistic trends was observed. There was no increase in the incidence of suicide, and no evidence of early mortality. In some patients vasomotor disturbances developed, while in others neurasthenia and depression states appeared. As time went on there were changes in body form and hair growth, height and weight, evidences of physical and psychic effeminacy, and less frequently premature senility along with climacteric disturbances. These appeared usually soon after age 40.

Artificial production of menstruation with ovarian hormones in cases of primary and secondary amenorrhea. Loeser, A., J. Obst. & Gynec. Brit. Emp. **41:** 86. 1934.

This is a brief report of 5 patients with primary amenorrhea treated by follicular hormone followed by corpus luteum hormone. Three of the subjects aged 25, 30 and 38 had previously never menstruated. On the 1st and 7th day 1 cc. of follicular hormone, 100,000 mouse units, was injected intramuscularly. On the 14th day 1 cc., 50,000 m.u., and on the 19th day a similar injection was given. On the 22nd to 26th day, inclusive, 1 cc. of corpus luteum hormone, 10 rabbit units, was injected. On the 28th day menstruation occurred.

The atrophic uteri enlarged and the patients were changed physically and psychically. In the other two cases results were negative but one of the patients had no endometrium and the other had tuberculosis. In a woman aged 80 arteriosclerosis was said to be improved and menstrual bleeding occurred. If the treatment was stopped only one menstrual bleeding occurred but sometimes recurred following the administration of follicular hormone alone. After several months of treatment apparently normal cycles were established. Details are omitted in this report and controls are not mentioned so one is left in doubt whether the author obtained better results than others have secured by administration of follicular hormone alone. The author states that in secondary amenorrhea menstruation may be produced with similar doses and that in most cases normal cycles follow cessation of treatment. Since no details are given there is some question whether the treatment was a causative agent. Without supporting evidence the author claims that the menstrual cycle of normally menstruating women can be altered either backwards or forwards.—J. P. P.

Therapy of dysfunctions of the ovary and the anterior lobe of the hypophysis (Eindrücke und Beobachtungen bei der Therapie der unregelmässigen Funktion der Ovarian und des Hypophysenvorderlappens). Louros, N., Arch. f. Gynäk. **153:** 296. 1933. Abst., Am. J. Obst. & Gynec. **28:** 622.

It is very difficult to evaluate hormonal therapy in ovarian and hypophyseal dysfunction because so many factors are involved. Observations on 700 patients with ovarian dysfunction have convinced the author that there are certain prerequisites for the success of hormonal therapy. The latter is valueless when disease exists in either the ovaries or the uterus. Only if the pelvic anatomy is normal should hormonal therapy be tried. The symptoms due to hypoovarianism yield promptly to this type of therapy, as do dyspareunia and sterility. Ovarian extracts to be efficacious must be given intravenously, as other methods are useless. The hypophyseal hormone should be given intramuscularly. Calcium therapy is a valuable adjuvant due probably to its effect upon the sympathetic nervous system.

Newer aspects of hypogonadism in the male. McCahey, J. F., Pennsylvania M. J. **36:** 295. 1934.

A great part of the newer knowledge of the endocrine function of the testes has developed from biologic investigations. If the pitfalls of the past are to be avoided, clinical application of this knowledge should be guided by biologic principles. We may expect a better understanding of the changes associated with hypofunction of the testes in the human based upon clinical and investigative study and free of popular fallacies. These studies should take cognizance of the two endocrine functions of the male gonads. The most practical problem at present is the correction of the pituitary-gonad hormone dysfunction in the early stages of enlargement of the prostate.—I. B.

The significance of the luteal action on the uterine muscle in the maintenance of gestation and initiation of parturition. Robson, J. M., J. Physiol. **81:** 372. 1934.

Experiments were undertaken by the author in order to study further the relation of the luteal secretion to the uterine changes and the factors responsible for controlling the development of uterine activity during the later stages of pregnancy. After the onset of pseudopregnancy (induced experimentally) rabbits were operated on and strips of uterus were removed and their reactivity to oxytocin was determined in the usual way, the minimum dose of hormone necessary to bring about a motor effect being accurately established. The results showed that although the cessation of pseudopregnancy is accompanied by a return of the uterine reactivity to oxytocin, the doses of hormone required to cause a motor effect are not particularly small, varying from 0.02 unit to a dose greater than 0.3 unit in 100 cc. of solution. The spontaneous rhythmic activity of the uterine strips removed in the early stages of pseudopregnancy (third of seventh days) is, on the whole, rather less than that observed later on (twelfth to fourteenth days). In 8 animals, the fetuses and placentae were removed at various stages of pregnancy, from the nineteenth to twenty-seventh days and the contraction of uterine muscle determined at subsequent periods. In no case was reactivity to oxytocin particularly high and

frequently it was very low. Results show that the increase in reactivity to oxytocin which occurs during normal pregnancy and culmination in parturition no longer takes place after removal of fetuses and placentae. The reactivity to oxytocin and spontaneous rhythmic activity of isolated strips of uterus were determined in rabbits: (1) at various stages following the removal of fetuses with or without the placentae during the later phases of gestation. In no case was cessation of luteal activity under any conditions (except normal pregnancy) associated with a rise in the reactivity of the uterine muscle similar to that observed at parturition. After a period of pseudopregnancy, it is found that the inhibition of the reaction to oxytocin observed during that period rapidly disappears, this process being dependent upon the cessation of secretion of corpus luteum hormone. The height of reactivity is never the same as that attained at parturition. The conclusion is drawn that in pregnancy, the luteal activity, in as far as the action on uterine muscle is concerned, is more prolonged than during pseudopregnancy, but nevertheless it does not extend to the end of gestation. Following removal of the uterine contents during pregnancy, the reactivity to oxytocin of uterine muscle is in all cases low compared with the sensitivity at parturition. The view is offered that during the later stages of gestation, the fetus ultimately controls the development of the reactivity of the uterine muscle to oxytocin and that the secretion of oestrin plays an important part in this effect.—L. Goldstein.

Histological changes in the mucous membrane of the genital tract of female cattle during the oestrous cycle (Untersuchungen über die histologischen Veränderungen der Schleimhäute des weiblichen Rindes im Brunstcyclus, ihr klinisch-anatomischer Ausdruck und ihr Vergleich mit den diesbezüglichen Verhältnissen bei den übrigen Säugetieren). Schreyer, W., Ztschr. f. Anat. u. Entwcklngsgesch. **100:** 40. 1933.

The epithelium and underlying connective tissue of the genital mucosa undergo marked characteristic changes during the cycle, which is, as a rule, 21 days in duration. Good photomicrographs illustrate the article. The data should be of practical value to those who are following the oestrous cycle of the cow.—A. T. R.

The treatment of sterility in cattle and cows by means of female sex hormone (Progynon) (Über die Bekämpfung der Sterilität bei Rindern und Kühen durch Sexualhormon [Progynon]). Steinach, E., Akademischen Anzeiger, No. 18. 1933.

Experiments on cows with a single dose of Progynon. For the experiment only those animals were selected in which the cause of sterility was purely endocrine. The injection was subcutaneously given on the neck, either in one dose or equally divided between the right and the left side. The latter method is to be preferred, as the conditions for the resorption are more favorable. No undue after-effects were observed. Forty-six animals were used. In 44 cases the result was positive: all the symptoms of heat could be observed, that is, vaginal and psychic symptoms. All animals were mated. The symptoms appeared, on the average, in 24 to 48 hours. The dosage of the single injection was 50,000 mouse units. In a few control cases with 30,000 mouse units, the result was equally positive but the effect was not so well pronounced as in the case of 50,000 units. The highest dose was 70,000 mouse units. The physiological effect of the injection is to reactivate the inactive ovary. Numerous cases of pregnancy (exact number is not given) proved the efficacy of the experiment.—G. L. Banay.

Removal of psychic and somatic effects of castration by means of male sex hormone (Proviron) (Behebung der psychischen und somatischen Kastrationsfolgen durch männliches Sexualhormon [Proviron]). Steinach, E. and H. Kun, Akadamischen Anzeiger, No. 18. 1933.

Experiments with the male hormone Proviron, prepared by W. Schoeller from male urine, Schering-Kahlbaum Laboratory, Berlin. The purpose of the experiments was to determine whether Proviron might activate the whole complex of male sex characteristics, psychic as well as somatic. As experimental material male rats were used which were castrated eight to ten months previously so that the atrophy of the sexual characteristics was well pronounced.

By repeated observations it was ascertained that the castrated males did not respond to the females. The hormone contained 25 capon units in a cubic centimeter of oily solution. Of this, 0.5 cc. or 12.5 capon units was injected daily. The injections were given from two to three weeks without any disturbance of the general condition. A protocol of one experiment is given. A rat was given 15 injections of 12.5 capon units in 18 days, 187.5 capon units altogether. The atrophic symptoms of the castration disappeared entirely. Libido reappeared on the thirteenth day, coitus two days later. Sexual response was maintained up to the eleventh day after cessation of injections.
—G. L. Banay.

Formation of corpus luteum is dependent on anterior pituitary lobe, and not on the maturing ovum. Fertilized ovum and hormones. Zondek, B., J. Physiol. **81:** 472. 1934.

Zondek's experiments were performed to indicate whether the ovum was involved in the formation of the corpus luteum. Rabbits were used as the experimental animals, although the number or condition of the animals is not mentioned. Ripe follicles were punctured, and the follicular fluid and ovum were aspirated. The fluid was centrifuged and the removal of the egg was demonstrated. In other cases, the projecting part of a follicle was clipped off at the time of removal of the egg. Other adjacent portions of the ovary were removed. Twenty-four hours after the operation an intravenous dose of pregnancy urine, prolan, 1000 R.U., was given. Examination 24-48 hours later demonstrated formation of the corpus luteum in the anovular follicle. In the discussion the author points out that, while the follicular ovum has no part in the hormonic events before fertilization, the increased production of hormones after fertilization is due, as he assumes, to an action of the fertilized ovum on the anterior pituitary. He points out, however, the exception that the testes of a stallion contain, by his tests, 4-5 times as much oestrogenic hormone as the human or mare placenta.—E. T. E.

Effect of combination of two antagonistic anterior pituitary hormones on sex organs of guinea pigs. Aff, Helen and L. Loeb, Proc. Soc. Exper. Biol. & Med. **31:** 957. 1934.

Two antagonistic hormones are present in the anterior pituitary gland, one causing growth and maturation of follicles and the other atresia of follicles in the ovary of the guinea pig; the former predominates in the anterior pituitaries of rabbit, rat and guinea pig and the latter in the glands of cattle, sheep and hog, at least in the state in which these latter are accessible for experimentation. The combined effect of these 2 antagonistic principles in the guinea pig was studied. Ninety-three guinea pigs were used. The investigation led to the following conclusions. The hormone causing atresia of follicles, administered in the first period, prevents the full effect of the hormone causing growth and maturation of follicles administered in a subsequent period. This inhibiting effect is in the same direction as the effect exerted by underfeeding, but it is much more pronounced. There is no antagonism between the effects of the atresia causing hormone and the process leading to the transformation of atretic follicles into interstitial gland and into pseudocorpora lutea, as far as the latter develop at the expense of the theca interna. We may assume that the atresia causing hormone fixes itself to the follicles, remains combined with them for some time and thus inhibits the full development and maturation processes in the follicles, which otherwise would have been induced by the follicular growth hormone. Previous observations were confirmed that the growth processes elicited in the vagina by the implantation of anterior pituitaries of rabbit and rat remain usually incomplete, although the number of mature follicles and of lutein bodies developing under those conditions in the ovaries may be much greater than in normal ovaries of guinea pigs at the time of ovulation.—Helen M. Aff.

Preparation and properties of an antithyrotropic substance. Anderson, Evelyn and J. B. Collip, Lancet, **1:** 784. 1934.

To a mare, the serum of which had been shown to have no inhibiting effect on thyrotropic hormone, there was administered for four weeks daily intravenous injections of this hormone. At the end of this period 2 cc. of the serum inhibited a dose of thyrotropic hormone ten times the minimal effective amount

for the hypophysectomized rat. The serum of this horse was administered in 1 to 2 cc. doses to six rats. The oxygen consumption rate gradually decreased until at the end of 18 days it was minus 16% of prediction. The experiment was controlled by injecting into 4 rats the serum from a horse which had received growth hormone and in 2 other cases of serum from a horse which was not injected. Neither of the control series showed comparable reductions in the oxygen consumption rate. The antithyrotropic substance was found to resemble insulin in its solubility. It proved to be heat labile. Various extracts of the antithyrotropic serum were injected into 18 rats that received simultaneously 100 unit doses of thyrotropic hormone. The effect of the latter was inhibited, as indicated by the oxygen consumption rate. In 3 hypophysectomized rats similar results were observed. The antithyrotropic principle failed to antagonize the action of thyroxine. Hypophysectomized rats which received the antithyrotropic substance in conjunction with thyrotropic hormone had a depressed metabolic rate associated with cellular activity in the thyroid gland. —R. G. H.

The pancreatropic substance in the pituitary (anterior lobe). Preparation and properties. Mode of action (Die pankreatrope Substanz aus dem Hypophysenvorderlappen; über die Darstellung und die Eigenschafter der pankreatropen Substanz. Die pankreatrope Substanz aus dem Hypophysenvorderlappen; über die Stoffwechselwirkungen der pankreatropen Substanz). Anselmino, K. J. and F. Hoffmann, Klin. Wchnschr. **12:** 1435; 1436. 1933.

Directions are given for the preparation of the pancreatropic extract of the posterior lobe of the hypophysis used by the authors in their previous report. Acetone dried gland, powdered, is extracted with water, the filtrate acidified with sodium acetate solution containing an excess of acetic acid (pH 5.2), the acidified solution ultrafiltered through collodion and neutralized. It is then ready for use, and is free by test of effects of the gonadotropic or thyreotropic or fat metabolism factors. Growth factor has not been tested for. The solution must be used in 2-3 days as it loses its activity on standing. With this preparation the authors have obtained the effects previously reported, namely, increase in size and number of the pancreatic islets with new islet formation. The authors administered their extract to dogs and rabbits and found a moderate lowering of blood sugar in both (a fall of 20 mg. per 100 cc. in one typical experiment reported). The maximum effect is seen about 2 hours after the injection. The extract decreases alimentary and adrenaline hyperglycemia. It is without effect on insulin hypoglycemia. In male rats the liver glycogen was markedly decreased concurrently with the fall in blood sugar. The extract is without effect on the fasting blood sugar or alimentary hyperglycemia of depancreatized dogs. The authors conclude that the anterior pituitary factor acts by way of the pancreas where it caused an increase in insulin output. Only typical experiments are reported. No data are given as to the number of animals used.—E. C. Albritton.

Anterior pituitary extract and glucose tolerance (Inyección de extracto anterohipofisario y tolerancia a la glucosa). Biasotti, A., Rev. Soc. argent. de biol. **10:** 36. 1934.

Administration of anterior pituitary extract (Evans and Simpson's) during several days into dogs produces a diabetic state. Injection of glucose (1 gm./kgm. intravenously) is not followed by a rise in R.Q. Hyperglycemia is more pronounced and prolonged than before the injections. Thyroidectomy does not modify this reaction. The extract diminishes the capacity for burning glucose.—J. T. L.

Absence of supposed hypoglycemic activity of blood from hypophysectomized dogs (Inexistencia de la pretendida acción hipoglucemiante de la sangre de los hipofisoprivos). di Benedetto, E., Rev. Soc. argent. de biol. **10:** 23. 1934.

Blood was taken from 16 dogs hypophysectomized 17 to 310 days previously and injected intravenously (10 cc.) into 14, subcutaneously (20 cc.) into 4, and intraperitoneally (20 cc.) into 8 rabbits. Blood from normal dogs was injected 5 to 6 days before or after as control. Blood sugar was estimated 12, and 3 hours after the injections. No significant increase in blood sugar was observed. Cowley's results were therefore not confirmed.—J. T. L.

The effect of an extract of the mammalian hypophysis upon the reproductive system of immature male salamanders after metamorphosis. Burns, R. K. and A. Buyse, J. Exper. Zoöl. **67:** 115. 1934.

Immature male salamanders (Amblystoma tigrinum Green), immediately after metamorphosis, were given continuous injections of an extract of mammalian hypophysis (sheep) for periods of 70 and 110 days. Precocious maturity of the entire reproductive tract was induced. Testes of experimental animals showed growth of 500 to 600% over uninjected controls reared under conditions otherwise identical. This growth was accompanied by spermatogenesis and development of accessory sex structures. Animals preserved after 70 days were approaching the climax of spermatogenesis, and the testes were largely filled with transforming spermatids. At 110 days, the peak of spermatogenesis had passed, and the gonads were filled with mature and degenerating sperm. In all gonads which had passed the peak of spermatogenesis retrogression occurred, consisting in shrinkage of the testis and degeneration and resorption of sperm. Retrogressive changes, however, were quickly followed by a rapid proliferation of residual spermatogonia, and a restoration of the germinal tissue, inaugurating a second spermatogenetic cycle close upon the first. Partial reduction of accessory structures followed retrogressive changes in the testis. In the testes of immature salamanders the effects of hypophysis stimulation are principally an increased proliferation of the germinal tissue, with precocious spermatogenesis. The accessory structures are affected secondarily. Males respond more rapidly to such treatment than females.

—Authors' Abst.

Inhibition of posterior pituitary hormones by serum of pregnancy (Hypophysenhinterlappen-sekretbindende Stoffe im Schwangerenserum). Dietel, F. G., Klin. Wchnschr. **12:** 1683. 1933.

A substance is reported to be present in pregnancy serum which adsorbs and inactivates the melanophore substance of the posterior pituitary. The finding is based upon the examination of 166 specimens of blood. The substance is increased in toxemia of pregnancy and at postpartum. It inhibits the melanophore substance obtained from human pituitary gland as well as from animal pituitary. If the serum is injected into the peritoneal cavity of the frog and the melanophore substance into the lymph sac no inhibitory effect is obtained. A study has been made of some of the physical properties of this substance. A substance is also found in pregnancy serum which destroys the antidiuretic principle of the posterior lobe. This was tested on male rabbits which were given 100 cc. water by stomach tube 10 minutes before injection of serum and pituitary extract. Normal human serum and pregnancy serum alone had no effect on water and chloride excretion. When normal serum was given in conjunction with posterior lobe extract the diuretic effect of the latter was diminished (report of 11 cases). It is concluded that a substance is present in normal serum which destroys the antidiuretic substance of the posterior lobe. This substance is increased in pregnancy (report of 24 cases) up to 20 times the normal amount; it begins to decrease by the fifth day post-partum. Evidence is presented to show that the antidiuretic inhibitory substance is not identical with the substance which inhibits the melanophore substance.

—Evelyn M. Anderson.

Lipoidgranulomatosis (type, Hand-Schüller-Christian); report of a case. Chester, W. and V. H. Kugel, Arch. Path. **14:** 595. 1932. Abst., Arch. Neurol. & Psychiat. **30:** 914.

In 1919, under the title, "Defects in Membranous Bones, Exophthalmos and Diabetes Insipidus: An Unusual Syndrome of Dyspituitarism," Christian reported the case of a girl, aged 5. Subsequently, instances of the disease were reported by numerous investigators. None of the cases except that of Hand came to autopsy. The unusual symptom complex was ascribed to dyspituitarism or to a tumor at the base of the brain. A case of lipoidgranulomatosis (type, Hand-Schüller-Christian) in a man, aged 31, with diffuse skeletal involvement, diabetes insipidus, dyspituitarism and extensive changes in the central nervous system, is described. Postmortem examination revealed: (a) lipoidgranulomatous lesions in the skull, femur, pituitary gland and lungs, and (b) increased vascularity in the anterior lobe of the pituitary gland, marked diminution in the number of eosinophilic cells, adenomatous hyperplasia and hypertrophy of the basophilic cells, a predominance of altered chromophobe cells and an increase in the stroma. The posterior lobe showed an increase in the

stroma, marked fibrosis and infiltration by round cells. The atrophy of the testes, with its attendant clinical picture, was subsequent to a primary lesion of the pituitary gland.

Migraine and hypophysis (Migraine et hypophysis). Drouet, P. L., L. Mathieu and L. Collesson, Rev. franç. d'endocrinol. **12:** 137. 1934.

The authors state that although the relationship between the hypophysis and migraine has been noted several times before, it was nearly always as a hypothesis and very few clinical or experimental facts had been contributed to support it. They therefore tried to contribute some such facts by examining the urine of patients with migraine, during and between attacks, for the posterior-lobe principle of the pituitary which causes expansion of the melanophores after injection into the frog. Fifteen patients with varying types of migraine were followed by means of almost daily morning urine specimens for periods of from 1 to 79 days—average 22 days. In a few instances the positive reactions in the frog were checked by the reactions of the erythrophores in the minnow (after Zondek and Krohn) which were also invariably positive. Results showed excretion of the pituitary principle in the urine of 13 of the 15 patients in a periodic manner, usually synchronous with the attack of migraine. The variations were: (1) Excretion of the principle before, during, and several days after the attack (this "overlapping" was the most common finding); (2) occasional isolated days during the attacks when the reaction was negative; (3) appearance of the principle only after the attack had started. Longer periods of excretion were associated with the severe attacks, shorter with the mild ones. In two patients with apparently typical migraine no positive reactions were obtained. Visual fields were tested in one patient and during the attack there was slight spherical contraction (10-20°) in both white and color fields. Therefore they advance the hypothesis that migraine may be due to hyperfunction of the pituitary with resultant congestion and increased intrasellar pressure. However, they also note the possibility that pituitary hyperfunction and migraine may both be secondary to a third common cause. The authors believe that they have definitely demonstrated changes in the physiology of the pituitary gland during attacks of migraine.—R. E.

The hypophysis and malignant tumors; the Aschheim-Zondek reaction in blastomas (Ipofisi e tumori maligni; il comportamento della reazione de Aschheim e Zondek nei blastomi). de Fermo, C., Arch. ital. di chir. **33:** 801. 1933. Abst., Am. J. Obst. & Gynec. **28:** 624.

The Aschheim-Zondek reaction was studied in 72 patients who had malignant tumors of genital and extragenital origin. The reaction was constantly negative for prolan B, and positive in 43% of all cases for prolan A. In patients with tumors of genital origin the reaction for prolan A was positive in 71.8%; in those of extragenital origin in 20%. The reaction was always negative in benign ovarian cysts, but positive for prolan A in 42.8% of uterine fibromyomas. The author concludes that the presence of prolan A is the expression of hyposexual function causing an increase in the activity of the anterior pituitary hormone.

The diverse views on the excretion of pituitary products in the case of teleost fishes (Les diverse voies d'excrétion des produits hypophysaires chez les Téléosteens). Florentin, P., Rev. franç. d'endocrinol. **12:** 271. 1934.

The similarity of the histological structure and function of all endocrine organs in all vertebrates is stressed. The histological structure of the pituitary of certain teleost fishes is described. The interstitial colloid present in the hypophysis is absorbed by two methods, viz., the capillary net in the anterior pituitary around the ventral nerve cells of the tuber and the cytoplasm of the nerve cells themselves. The author concludes that the pituitary functions similarly in the lower vertebrates as in mammals. It may be said with certainty that during certain time of the year (cyclic sexual activity) in the lower vertebrates, the vegetative nuclei of the cinereal tuber and the cavity of the third ventricle are the depositories of the active principle secreted by the hypophysis. The effect of this pituitary substance on other organs may be discovered through the combination of histological and physiological research.
—G. Ayers.

The hypophyseal growth hormone and glutathione concentration; does the hormone influence the concentration concurrently with the stimulation of increase in weight? Gregory, P. W. and H. Goss, J. Exper. Zoöl. **69:** 13. 1934.

Two experiments were performed with adult female rats which had reached growth stasis under normal conditions. The rats were divided into two groups of approximately equal weights. The experimental groups received subcutaneous injections of 1 cc., of the hypophyseal growth hormone daily, for 15 days. One group of the control rats was not injected, while the second group of control rats was injected with a heat inactivated solution of the hypophyseal growth hormone. The growth hormone injected rats showed marked increase in weight, while the control rats did not increase in weight. After 15 days of injection, both the experimental and the control rats were killed. The whole liver and a sample of muscle from each rat were analyzed separately to determine the total iodine reducing substances present in these tissues. In the second experiment, both the amount of the ascorbic acid fraction and the glutathione fraction were obtained, in addition to the total iodine reducing value. The ascorbic acid fraction present in the tissue filtrates of the experimental groups was approximately the same as that of the control groups. The evidence from these experiments indicates that concurrently with an increase in weight induced by the hypophyseal growth hormone, there is an increase in the glutathione concentration of the muscle.—Authors' Abst.

Growth and regeneration of tissue in frog tadpoles following the administration of an extract of the anterior pituitary gland. Herrell, W. E., Anat. Rec. **59:** 47. 1934.

Frog tadpoles are markedly influenced by the extract of the anterior pituitary gland. Large tadpoles have been produced by its administration. These animals deviate from the normal as regards shape, body contour, general appearance, and degree of pigmentation. Regenerating tissues are also influenced by the extract. The effect depends partly on the phase of regeneration as related to the time of extract administration. If the extract is administered before, at the time of, or shortly after injury, the process is retarded. Extract administration during the proliferative phase offers a definite stimulus to the speed as well as amount of tissue regeneration. "Form regulation" is also influenced during this period. Extract administration during the differentiative phase is followed by overgrowth of tissues. Comparison with the effects of thyroid extract on regenerating tissues (Speidel, 1929) indicates dissimilarity in the proliferative and differentiative phases. Similar effects, however, are shown by both in hindering regulation. This is not permanent for pituitary as it is with thyroid extract. Results cited, and recent work quoted, indicate that pigment metabolism is under the control of the pituitary-thyroid mechanism, the integrity of which must be maintained under normal conditions.
—Author's Abst.

Spleen in hypophysectomized dogs (El bazo de los perros hipofisoprivos). Houssay, B. A. and J. M. Lascano-González, Rev. Soc. argent. de biol. **10:** 399. 1934.

The weight of the spleen in hypophysectomized dogs is not significantly different from that of normal animals. In hypophysectomized puppies it was smaller than in controls, but its size was proportionate to the total body weight. The Malpighi corpuscles are more numerous and larger than in the controls in the adult animal; in puppies they are more numerous but no larger.—J. T. L.

The influence of the hypothalamus on pituitary activity. Second communication. Karplus, J. P. and O. Peczinik, Arch. f. d. ges. Physiol. **322:** 402. 1933.

The authors report three different sets of experiments. Using their own operative technic (reported in their first communication) the hypothalami of cats were exposed under ether anesthesia by trephine openings in the floor of the skull. The hypothalamus was stimulated by electricity. Specimens of the cerebro-spinal fluid were obtained from each animal from the IV ventricle by needle puncture of the occipital membrane (cisterna puncture). Specimens of the fluid were obtained just prior to hypothalamic stimulation (pre-excitation fluid) and after hypothalamic stimulation (post-excitation fluid). For the first set of experiments 6 adult cats were operated upon. Three minnows were used for each experimental cat. Each set of minnows were injected subcu-

tanously with pre-excitation fluid in amounts from 0.2 to 0.3 cc. Minnows injected with fluid from 2 cats caused only a slight "Intermedin" reaction (bright red pigmentation in underside of fin attachments which quickly faded). The same sets of minnows were then injected with specimens of post-excitation fluid obtained from their respective cats from 1.5 to 4 hours after hypothalamic stimulation. Minnows injected with fluid from 2 cats produced a maximal "Intermedin" reaction. This reaction remained positive for 12 hours after injection and then began to slowly fade. Three cats gave a strongly positive reaction and one cat only a weak reaction. However, the reactions of the post-excitation fluid completely overshadowed the slight reactions obtained with pre-excitation fluid. Additional control experiments were performed by injecting some other minnows, subcutaneously with physiological saline, Ringer's and Tyrode's solution, all of which yielded no reactions. Finally some other minnows were injected with Pitraphorm (Schering) in dilutions of 1:10 and 1:20 in 0.2 cc. amounts with which, in every instance, the maximal reaction was obtained. In the second series of experiments, five large adult cats were operated upon as in previous experiments. After hypothalamic stimulation, specimens of their ventricular fluid were obtained as above. Each specimen of post-excitation fluid was injected intravenously into a small cat the carotid blood pressure of which was recorded by the mercury manometer. Post-excitation fluid (0.8 to 1.0 cc.) in 4 cases gave a slight but distinct and relatively lasting increase of blood pressure. The blood pressure curves were similar to and equivalent to those obtained with dilutions of the standard Schering's Pitraphorm preparations. In the fifth case only 0.4 cc. of post-excitation fluid was obtained; this had no effect in the smaller cat in this amount.

—W. Freemen.

The effect of extract of pregnancy urine upon hypopituitarism in the male. Kunstadter, R. H. and L. S. Robins, J. Pediat. **4:** 774. 1934.

Favorable results are reported in cryptorchidism and genital hypoplasia following the administration of antuitrin-S. There was no stimulation of weight loss and no alteration in the typical distribution of fat. In seven of the eight cases there was an increase in growth above the normal. The basal metabolic rate was lowered below the lower limits of normal in four of five cases. There was a tendency for the sugar tolerance to decline. The treatment consisted of injection of antuitrin-S 100 R.U. three times a week for periods of from three to six months.—M. B. G.

Clinical studies in diabetes insipidus. First report. Diabetes insipidus, water and salt regulation and their behavior under the influence of Novasurol and pituitary extracts (Etudes cliniques sur le diabète insipide; diabète insipide, hydro- et chlororégulation et leur comportement sous l'influence du novasurol et des extraits hypophysaires). Landau, A. and J. Wajsman, Rev. belge sc. med. **6:** 273. 1934.

This somewhat lengthy paper begins with a historical review of the concepts of the nature of diabetes insipidus with special reference to the parts played by the pituitary body and the diencephalic areas. The authors accept Veil's division of the cases into hypochloremic and hyperchloremic types. They report a typical case of idiopathic diabetes insipidus and show the anti-diuretic effect of low salt diet, intranasal pituitary extract, Novasurol (organic mercurial diuretic) and hypodermic use of pituitary extract. From salt and water studies they conclude that the Novasurol acts primarily on the kidneys while pituitary extracts affect the tissues directly. There is little in this paper that is not already well known in the literature of the subject.—S. Silver.

Occurrence of follicle stimulating and luteinizing factors of anterior pituitary-like principle in urine of women castrated by roentgen treatment or by operation. Lassen, H. C. A. and E. Brandstrup, Acta obst. et gynec. Scandinav. **14:** 89. 1934. Abst., J. A. M. A. **102:** 2074.

In a series of women who had been subjected in 36 instances to roentgen castration and in 10 instances to surgical castration, Lassen and Brandstrup made a total of 436 tests for anterior pituitary-like principle in the urine. The tests were made once a month for from three to 31 months. With the technic employed, anterior pituitary-like principle could be demonstrated only when 1 liter of urine contained in excess of 400 mouse units. In the women who had

been castrated with roentgen rays, the reaction for the follicle-stimulating factor of anterior pituitary-like principle was positive in approximately 30% of the urine specimens (343) examined. The reaction for the luteinizing factor of anterior pituitary-like principle was positive in 7% of the specimens. The incidence of the reaction for the former factor was practically the same throughout the observation period but the reaction for the latter factor was positive most frequently during the first half year after castration. Of the roentgen castrates, 92% excreted the follicle-stimulating factor of anterior pituitary-like principle in amounts of over 400 mouse units per liter at some time during the observation period, while a similar amount of the luteinizing factor of anterior pituitary-like principle was detected in 44% of the cases. In the surgical castrates a positive reaction for the follicle-stimulating factor of anterior pituitary-like principle was obtained in about half the urine specimens examined, while the reaction for the luteinizing factor of anterior pituitary-like principle was positive in about 9% of the specimens. The reactions for anterior pituitary-like principle are most frequent during the first six months after the operation. All 10 patients excreted at some time or other after the operation the follicle-stimulating factor of anterior pituitary-like principle in amounts of more than 400 mouse units per liter of urine, most of them within the first half year after the operation. Six patients gave a positive reaction for the luteinizing factor of anterior pituitary-like principle within that period. In suitable control material less than 15% of the cases gave positive reactions for the follicle-stimulating factor of anterior pituitary-like principle and the reaction for the luteinizing factor of anterior pituitary-like principle was positive in from 1 to 2% of the cases. Tests were made also (one in each case) on 63 women of the menopausal age, and the reaction for the luteinizing factor of anterior pituitary-like principle was positive in 14% of the cases.

Chorionepithelioma; early diagnosis by the quantitative determination of anterior pituitary-like principle from the urine of pregnancy. Leventhal, M. L. and W. Saphir, J. A. M. A. **103:** 668. 1934. Abst., A. M. A.

The authors present a case illustrating the importance of determining quantitatively the amount of anterior pituitary-like substance in the urine following the expulsion of a hydatidiform mole. Although histologically the tumor was not the most malignant type of chorionepithelioma (choriocarcinoma), still the high gonadotropic concentration as a biologic indicator points to the potentiality of profound malignant change. This is important because the histologic picture is not always a true prognostic index in chorionepithelioma. This fact was well demonstrated in the unusual case recently reported by Lackner and Leventhal, in which, microscopically, a typical choriocarcinoma was found, and, in spite of both pulmonary and vaginal metastases, cure (the patient is now well after eight years) followed hysterectomy and high voltage roentgen irradiation. It will be interesting, in the future, to note whether the amount of anterior pituitary-like principle bears any relation to the histologic classification of the choriomas (as set forth by Ewing). The performance of a radical operation entirely on the basis of a laboratory observation of 333,000 mouse units of gonadotropic substance per liter of urine and in the absence of any palpable or visible pathologic change was justified by the discovery of a potentially malignant tumor in an early stage of its proliferation. In the absence of clinical manifestations, an amount of gonadotropic substance in the urine in excess of 20,000 mouse units per liter indicates an early chorionepithelioma. The disappearance of a positive Aschheim-Zondek test following operation is a true indication of the absence of live chorionic villi (recurrence or metastasis). Therefore the use of postoperative radiation therapy is unnecessary in the presence of a vegetative test. This would obviate the deleterious effects of irradiation in cases in which it is not needed.

The pseudo-hypophyseal infundibulotuberian syndrome (Syndrome infundibulotubérien pseudo-hypophysaire). Lhermitte, J. and P. Pagniez, Presse. méd. **42:** 649. 1934. Abst., Surg., Gynec. & Obst. **59:** 332.

The authors report the case of a woman forty-two years of age who came to the hospital because of diabetic symptoms—polyuria, polydipsia, glycosuria, hyperglycaemia, obesity, dysmenorrhoea, and irregular menstruation. She stated that during infancy she had suffered from poliomyelitis and from encephalitis of undeterminable origin. The authors believe that the encephalitis

was due to the poliomyelitis virus. At the age of forty years the patient developed facial paralysis with crossed hemiplegia. On the basis of the history and the roentgenographic demonstration of enlargement of the sella, a hypophyseal syndrome was considered. The patient died of acute apoplexy. At autopsy the hypophysis and sella were found to be absolutely normal, but the hypothalmic centers appeared on gross examination to be markedly altered by an infectious and degenerative process. The findings of autopsy and of histological examination of the brain, especially of the infundibulum and mesencephalon, are described in detail. The authors contend that this syndrome should be termed the "hypothalamic syndrome" or "infundibulotuberian syndrome" rather than the "hypophyseal syndrome." They state that the hypothalamus is considered to be a regulator of fat, carbohydrate, and water metabolism.

The effect of operative procedures on the anterior lobe of the pituitary on the metabolism of depancreatized dogs (Der Einfluss von operativen Eingriffen am Hypophysenvorderlappen auf die Stoffwechsellage des pancreasdiabetischen Hundes). Lucke, H., E. R. Heydemann and O. Berger, Ztschr. f. d. ges. exper. Med. **92:** 711. 1934.

A study has been made on the relationship of the anterior pituitary to diabetes. By destroying the anterior pituitary the carbohydrate metabolism of the depancreatized dog is strikingly improved. Animals which required considerable doses of insulin to prevent diabetic coma no longer require any insulin after destruction of the anterior pituitary. Typical cases are reported from a large series of experimental animals. Following pancreatectomy on a dog the fasting blood sugar was stabilized between 400-450 mg. per cent with diet and 16 units insulin per day. Fourteen days after the pancreatectomy, a second operation was performed under ether anaesthesia, in which the pituitary gland was destroyed by electric needle. The insulin injections were stopped 24 hours before the second operation. Immediately following the destruction of the pituitary, the blood sugar rose to 800 mg. per cent. In four hours it had fallen to 520 mg. per cent. On the morning of the first day postoperative it was 420 mg. per cent. The urinary sugar was also diminished. A second case is described in which there occurred a striking improvement in carbohydrate tolerance in the depancreatized dog following destruction of the pituitary gland. In a third case reported the operation on the pituitary was not performed until coma was imminent. In this case the animal died. The blood sugar may fall to an hypoglycemic level after the second operation. This was shown in the fourth case reported in which the blood sugar fell from 575 mg. per cent immediately after operation to 40 mg. per cent in approximately 24 hours. It is shown that extracts of anterior pituitary will cause a greater rise in blood sugar in the depancreatized hypophysectomized animal than in the normal. Hypophysectomy causes an increased sensitivity to insulin in the depancreatized animal.—Evelyn M. Anderson.

The effect of hypophysectomy on the growth of the Walker rat tumor. McEuen, C. S. and D. L. Thomson, Brit. J. Exper. Pathol. **14:** 384. 1933.

Twenty-six rats bearing tumors 10 to 12 days old were matched in pairs and the hypophysis was removed from one member of each pair. It was found at autopsy 21 to 36 days later that the tumors had increased more slowly in size in the hypophysectomized than in the normal rats. In a subsequent series of 8 rats in which the pituitary extirpation was verified at autopsy similar results were secured. In 15 rats transplants of the tumor were made from 7 to 19 days after hypophysectomy. These transplants grew abnormally slowly. The total weight of the tumor-bearing rats remained constant after hypophysectomy but that of the controls increased.—R. G. H.

Studies on the physiology of lactation; the assay of the lactogenic hormone of the anterior hypophysis. Nelson, W. O., Anat. Rec. **60:** 69. 1934.

The various methods which have been suggested for the assay of the hormone are discussed. These include the pigeon crop-gland, pseudo-pregnant rabbit, and spayed guinea-pig tests. In the present study 137 of 158 adult spayed guinea-pigs lactated spontaneously soon after ovariectomy. On this basis the recently spayed guinea-pig is judged untrustworthy for lactation assays. About 300 normal adult females and 35 males bearing ovarian grafts

were injected with various lactogenic extracts. Although lactation may be induced readily in these animals reasons are given for eliminating them as assay subjects. Finally 78 female guinea-pigs which had been hysterectomized during the latter half of pregnancy were injected with lactogenic extracts and successfully employed as test animals. Their well-developed mammary glands, prepared for lactation, serve to recommend them together with the pseudo-pregnant rabbit as standard subjects for the assay of the lactogenic hormone. —Author's Abst.

The pituitary of Jenynsia lineata (La hipófisis de Jenynsia lineata). Rojas, P., L. Castellengo and M. E. Silva Alcorta, Rev. Soc. argent. de biol. **10:** 414. 1934.

A microscopical description of the gland in this teleostean. Great individual varieties were observed. There is constanly a chromophil lobe in the anterior part and a chromophobe lobe posteriorly; in one case in 16 a transitional part between the two was seen. The pars nervosa descends from the tuber cinereum and forms digitations within the chromophobe lobe, and in a lesser degree within the chromophil lobe. The vascular sinus is not very pronounced. In gravid females the chromophil lobe cells are intensely vacuolized and increased in size.—J. T. L.

The water and solid content of the brains of albino rats treated with the growth hormone. Rubinstein, H. S. and L. M. Fox, J. Comp. Neurol. **60:** 349. 1934.

Thirteen adult male white rats (Mus norvegicus albinus) were divided into five test animals and two sets of controls containing four animals each. All were subjected to the same diet and surroundings. The test animals received daily (except Sunday) intraperitoneal injections of 2 cc. of an alkaline aqueous extract of the anterior lobe of beef pituitary glands. One set of controls was injected likewise with a beef extract prepared similarly to the pituitary extract. The other control group remained uninjected. Although injections were given for 22 weeks, differential growth occurred only during the last 14 weeks, at the end of which time the test animals had gained significantly in body weight over the controls. All animals were then sacrificed and their brains weighed and subjected to desiccation. From a statistical analysis of the data it was concluded that (1) the growth hormone fails to increase the size of the brain in proportion to the body; (2) the water and solid contents of the brains of animals artificially enlarged by the growth hormone retain their normal proportions.—Authors' Abst.

Experimental investigations on the removal of the hypophysis from rabbits and the consequences (Experimentelle Untersuchungen über die Exstirpation der Hypophysis bei Kaninchen und deren Folgeerscheimungen). Saito, G., Folia endocrinol, japonica **10:** 13. 1929.

This is a summary of some work done during 1923-1924. The removal of the pituitary is effected by the nasal approach. This avoids bleeding and cerebral injury. If the whole pituitary or the anterior lobe be completely removed cachexia results. Partial removal disturbs growth, gonad development, causes fat deposition in the whole body, stops the development of the secondary sexual characteristics and reduces bodily resistance. The eosinophiles in the anterior pituitary apparently have a special influence on the growth of animal bodies; on the contrary the basophiles do not. The mother cells of both attain only a young form. Partial removal of the anterior pituitary influences other endocrine glands such as the thyroid and causes fatty degeneration of the thymus. The interstitial cells of the gonads are affected. —G. B. Ayres.

Hypophysis and castration obesity (Hypophyse und Kastrationsfettsucht). Schultze, K. W., Arch. f. Gynäk. **115:** 327. 1934.

The observation of Anselmino and Hoffmann that extracts made from the anterior pituitary lobe affect the ketone bodies of the blood is confirmed. Injection of such extracts in the rat is followed by an increase of the ketone bodies the maximum of which is reached within 3 hours after injection. This "fat metabolism hormone," present in pituitary extracts and demonstrable with the aid of the rat test, makes its appearance also in human blood following the ingestion of a fat meal. Control experiments made on healthy young women during their intermenstrual interval showed that the maximum

effectiveness of their blood plasma in the rat test was obtained within two to three hours after ingestion of 100 cc. of olive oil. The rat test was applied to the study of four cases of castration obesity. All four patients received a test meal of 100 cc. olive oil. Blood plasma obtained from these patients following the test meal produced the same rise of ketone bodies in the rats as elicited by the control blood samples. Hence production of the fat metabolism hormone does not seem to be decreased in castration obesity and the latter cannot be accounted for by pituitary insufficiency.—M. Goldzieher.

Cytological studies on the rat pituitary after injections of pregnancy urine extract and pregnancy blood serum. Severinghaus, A. E., Anat. Rec. **60:** 43. 1934.

Leonard (1933) demonstrated that after P.U. injections into male and female rats the gonad-stimulating potency of their pituitaries was decreased. Certain pituitaries from Leonard's injected rats are here described, together with some additional material. P.U. injections into normal immature females result in degranulation of the pituitary basophiles, and transformation of many chromophobes into small basophiles lacking in typical granules. Increased mitochondria, and a much-hypertrophied Golgi apparatus indicate an active secretory condition of these cells. Basophilic degranulation occurs also in adult injected females, but newly formed basophiles are fewer. There is marked regression of the acidophiles. When adult males are injected, basophilic degranulation occurs to a lesser degree. Long, heavy injections may encounter a refractory stage in the animal, after which pituitary changes not only fail to proceed, but actually revert to normal. Pituitaries of injected castrates of either sex could not be distinguished histologically from their castrate controls. Since basophilic degranulation and reduced gonad-stimulating potency are constant, coincident features, it is additional evidence that basophiles are the source of the gonadotropic hormone. The degranulated basophile, identified by its characteristic Golgi apparatus, is also found in the pituitary of pregnancy, and is the so-called "pregnancy-cell." The variation in cytological findings indicates the importance of such data as age and sex, as well as the potency, dosage and duration of injections.—Author's Abst.

Hypophyseal infantilism; treatment with an anterior hypophyseal extract. Preliminary study. Shelton, E. K., L. A. Cavanaugh and H. M. Evans, Am. J. Dis. Child. **47:** 719. 1934.

Six children believed to have a secretory deficiency of the anterior hypophysis have been studied over a period of two years. The presumption was verified in one girl in whom retarded growth followed curretage of the pituitary area for a supracellar cyst. Four were treated by intragluteal injection of a growth-stimulating fraction of the bovine anterior hypophysis. One was treated by another method. One was left untreated for 23 months and then given the growth-stimulating hormone. The last two patients serve as controls. The author concludes that growth has been stimulated by the injection of a growth-stimulating fraction of the anterior hypophysis. The substance produced changes in the facial expression and configuration of a dwarfed child. In its present potency and by the present method of administration the preparation will not rehabilitate one who has fallen far behind in development unless it is given in massive doses and over a prolonged period of time. It may be of clinical value in certain cases of mildly arrested development and in the prevention of hypophyseal infantilism.—M. B. G.

Prolan in the pituitary (Prolan in der Hypophyse). B. Zondek, Klin. wchnschr. **12:** 22. 1933.

Pituitaries of human beings and of cattle were studied for the localization of the production of the sex-stimulating hormones. The posterior lobe was divided into 3 parts and pieces from 20 to 200 mg. were implanted into infantile female mice. The infundibulum and parts of the floor of the brain in this region were studied in a similar manner. Twenty-four human glands and 20 from cows were used. Results showed that the posterior lobe of cattle pituitaries were free from prolan but a slight amount was present in that of human beings. A slight amount was in the infundibulum in both cases. Brain tissue contained no hormone. The author believes that the basophile cells of the anterior lobe produces prolan.—S. L. Leonard.

The inactivation of insulin by normal and diabetic blood. Black, P. T., Brit. J. Exper. Path. **14:** 318. 1933.

Insulin incubated with serum or with laked cells or with cytolysed leucocytes was injected subcutaneously into 8 fasting rabbits. Insulin incubated with laked cells was shown in 13 rabbits to have lost part of its power to lower the blood-sugar. This loss was ascribed to destruction of the insulin by an enzyme other than trypsin. The inactivated substance does not readily pass from the cells to the plasma. Insulin incubated with heparinized whole blood retained its power to lower the blood-sugar when injected intravenously into rabbits. This was also true of insulin incubated with intact red cells or leucocytes. Blood from a diabetic patient, displaying resistance to insulin, had no more than normal power to destroy insulin. This investigation therefore does not support the view that the symptoms of diabetes are due to destruction of insulin in the circulation.—R. G. H.

Chronic abdominal splanchnopathy and diabetes mellitus (Splancnopatie addominali croniche e diabete mellito). Cannavò, L., Clin. med. ital. **64:** 999. 1933. Abst., J. A. M. A. **102:** 253.

Cannavò describes a form of diabetes mellitus secondary to chronic abdominal splanchnopathy, which he found in a number of patients at an earlier age than that of hypertonic diabetes, the diabetic symptoms beginning between 30 and 40; it is not accompanied by arterial hypertension and is a genuine pancreatic diabetes. In the beginning period it shows a notable diminution of external pancreatic secretion. It occurs in patients presenting a long history of dyspeptic symptoms, resulting from chronic appendicitis, cholecystitis or peptic ulcer. The development of diabetic symptoms is always preceded and accompanied by classic pancreatic symptoms. Roentgenologic examination generally reveals enlargement of the pancreas and marked changes in form of the serous membrane that surrounds and envelops it. Histologic examination demonstrates the presence of interstitial pancreatitis with lesions of the secretory cells of the pancreas. Hereditary diabetes and etiologic factors, such as syphilis, malaria, typhus and epidemic parotitis, which often influence the genesis of true diabetes, were not present in this form of the disease.

Increased carbohydrate tolerance in diabetics following the hourly administration of glucose and insulin over long periods. Ellis, A., Quart. J. Med. **3:** 137. 1934.

Because of the possibility that instability of the blood sugar occurring in certain diabetics under treatment with insulin might be due to depletion of the hepatic glycogen reserves, Ellis conceived the idea of administering glucose and insulin hourly over long periods of time. After a few days' observation in hospital on diet and insulin, the patients were kept in bed and all food except glucose withheld. Anhydrous glucose dissolved in a few ounces of water was then given hourly during the day and night in doses of from 10 to 30 grams. Insulin was given subcutaneously hourly throughout the 24 hours in doses of 1 to 10 units. Blood sugar estimations on capillary blood were made at hourly or two-hour intervals during the whole period of observation, using the Folin-Wu micro technique. The period of glucose administration varied in different patients from two to 16 days. In the eight cases reported there occurred, with one exception, either during or following the period of glucose administration, a definite improvement in carbohydrate tolerance, indicated by a diminution in the amount of insulin required to keep the blood sugar normal or subnormal. With two exceptions, the subsequent history of the patients does not suggest that they were permanently either benefited or harmed by the treatment. The quantity of glucose given to these patients is greater than in any previous observations on diabetes, approximating 600 gm. daily. The patients exemplified severe cases of diabetes, the larger amounts of glucose given were well tolerated and the insulin required was no greater than that necessary on an ordinary restricted diabetic diet. During and following such administrations of glucose marked temporary improvement in carbohydrate tolerance with great reduction in the amount of insulin required sometimes occurred. The results are shown in a series of charts. The possible bearing of these observations on the theories of the pathogeneses of diabetes is discussed, although in the final analysis the author finds it impossible to satisfactorily explain the meaning of the diminished insulin requirement.

—H. C. Shepardson.

Subtotal resection of the pancreas for hypoglycemia. Graham, E. A. and A. F. Hartmann, Surg. Gynec. & Obst. **59:** 474. 1934.

The case is reported of a baby one year of age from whom 80% to 90% of the pancreas was removed in the hope of correcting chronic hypoglycemia with repeated convulsions and marked retardation in mental development. After operation the blood sugar soon stabilized at practically normal level. All symptoms of hypoglycemia promptly disappeared and have not recurred. Marked improvement was noted in the mental and physical condition of the patient. The pancreatic tissue that was removed appeared to be normal. No interference with the general nutrition or the acid-base balance of the blood followed the operation.—R. G. H.

High carbohydrate diets and insulin efficiency. Himsworth, H. P., Brit. M. J. No. 3836. P. 57. 1934.

During the last four years the use of diets containing a relatively large quantity of carbohydrate has become more and more common in the treatment of diabetes mellitus. When first introduced, these diets were received with many theoretical objections, but their undeniable success rapidly compelled serious consideration and encouraged increasing acceptance. Himsworth felt that the divergence between theoretical objection and practical success might be in part, at least, explained by the influence diet played upon the sugar tolerance of healthy subjects and the relation of sugar tolerance and insulin sensitivity. He therefore determined to investigate the result of different dietetic conditions both on sugar tolerance and on sensitivity to insulin in healthy young men varying in age from 18 to 22 years. Two glucose tolerance curves were obtained on the same subject; one after having followed a high-carbohydrate low-fat diet and a second after a low-carbohydrate high-fat diet (both diets having equal caloric values). Actual figures for the diets are not included in this report. For the insulin depression curves, blood samples were obtained in the resting state then following the intravenous injection of 5 units of solution of crystalline insulin, at intervals first of every 2 minutes and later every 3 minutes for 35 minutes. Such curves were obtained while the individual was following, first a high-fat diet and second an equicaloric high-carbohydrate diet. The results of two such experiments are shown in two graphs. The author feels that his results assist in explaining Allen's glucose equivalent of insulin in that the giving of carbohydrate raises the efficiency of both injected and pancreatic insulin in the normal subject. If the action can be shown to be the same in diabetic individuals Himsworth feels that the improvement of the latter patients on a high-carbohydrate diet is to be ascribed not to the greater stimulation, and consequent overstrain, of their insulin-secreting tissue by the excessive intake of carbohydrate, but rather to the rendering of the diabetic more susceptible both to his own and the injected insulin. He finally suggests the possibility of the existence of a type of diabetes due not to diminished secretion of insulin by the pancreas but to a greater or less impairment of the organism's susceptibility to insulin.—H. C. Shepardson.

Insulin in treatment of peptic ulcer. Jones, C. R., Am. J. Digest. Dis. & Nutrition, **1:** 135. 1934. Abst., J. A. M. A. **102:** 2141.

Jones administered from 10 to 15, sometimes 20, units of insulin subcutaneously 15 minutes before eating to 12 patients having recent peptic gastric ulcers, 6 patients with recurring ulcers and 8 patients presenting complicated ulcers in the sense of Troitzky's theory. The action was not immediate, but usually in from 4 to 5 days of insulin treatment there was a decrease of pain, improvement in the subjective condition, and an increase in weight, which was coincident with a decrease of the vagotonus. In 7 of the 12 patients of the first group, pain disappeared after 5 days and the blood from the contents of the gastro-intestinal tract after 7 days, in spite of the distinct increase of the gastric secretion. In 4 persons the symptoms disappeared in from 6 to 10 days and in one patient the feeling of complete well being appeared only after two weeks. The weight of all patients increased from 3 to 8 pounds (1.3 to 3.6 Kg.). In the second group of 6 patients, insulin treatment (from 20 to 30 units daily), carried out for two to three weeks, gave good results, so that roentgen examination revealed no signs of the previously existing ulcer. Four of the patients were observed for 9, 11, 13 and 15 months, respectively. No recurrences were observed. In one patient there was a recurrence after

five and one-half months; the ulcer symptoms disappeared again after 10 days of insulin treatment. The last group of patients experienced undoubted relief under the influence of from 14 to 16 days of insulin treatment: the pains, although considerably lessened, continued to recur from time to time, especially in the periods of nondigestion, apparently correlating to movements of the stomach. Nevertheless, the patients stated that their well being was improved greatly. They gained in weight and had good appetites; 2 of them, after leaving the hospital, could do physical work of which they had been incapable before.

Utilization of galactose and effects of insulin (L'utilizzazione del galattoso nei cani ed azione dell'insulina). de Lucia, P. and M. Torella, Arch. di sc. biol. **17:** 422. 1932. Abst., Physiol. Absts. **18:** 582.

Galactose was given to dogs by the mouth, subcutaneously, and endovenously, and it was found that in all cases when 1 gram per kg. was given about 75% was retained. This percentage was not affected by injection of insulin, nor was galactose found capable of alleviating the symptoms of hypoglycemia produced by the injection of insulin.

Diabetes mellitus in children. Newcomb, A. L., J. Pediat. **4:** 617. 1934.

This is a report of a study of 65 children with diabetes mellitus: 63% were males and 37% females. Scandinavian nationalities predominated. A history of diabetes was found in the immediate family or ancestors in 21%. Infection and overfeeding were important factors in the production of an acute onset. Glucose tolerance remained above 100 gm. unless severe infection or prolonged dietary indiscretions occurred. Death occurred in 5 children in coma.—M. B. G.

A statistical evaluation of different methods of the detection of arteriosclerosis in diabetes mellitus. Rabinowitch, I. M., W. L. Ritchie and H. S. McKee, Ann. Int. Med. **7:** 1478. 1934.

The purpose of this study by Rabinowitch and his co-workers was to determine which of the various methods for the detection of arteriosclerosis in the diabetic gives the most information and to evaluate the methods statistically. Mortality and morbidity data clearly indicate that cardio-vascular disease is one of the most important contributing factors in the high death rate from diabetes mellitus. Furthermore arteriosclerosis develops in the diabetic patient at a much younger age than in normal individuals. It is therefore not only appreciably affecting mortality among adults but sooner or later, if not controlled, will exert the same effect among children. The methods studied by these workers include (1) clinical examination—using as a basis four suggestive signs of arteriosclerosis, namely, thickened radial vessels, hypertension, enlargement of the heart and accentuation of the aortic second sound; (2) examination of the occular fundi; (3) Roentgen-ray examination of the heart and liver extremities. For the study 1500 diabetics were selected at random. Each method was studied individually and then in different combinations with each other whenever the necessary information was available. The results are set forth in various tables. One especially, that giving the incidence of cardiovascular disease among 1500 diabetics according to a combination of three methods of diagnosis, is extremely interesting. It indicates that regardless of which three methods are used the incidence of arteriosclerosis is found to be 55% to 60%. These data agree very closely with postmortem data. It is clearly shown that the incidence of vascular disease is quite high and that by a combination of methods vascular disease is more readily detected than by the use of any one method to the exclusion of all others. Finally emphasis is laid on the necessity of early diagnosis for adequate prevention and successful treatment.—H. C. Shepardson.

Insulin in undernourished non-diabetic children; attempt to induce gain in weight. Radwin, L. S. and S. S. Brown, J. Pediat. **4:** 315. 1934.

The results obtained in a study of 11 boys in an orphan asylum ranging in age from 11 to 14 years of age are reported. They were all underweight from 4 to 20% below the accepted standards for their height and age. The authors conclude that insulin given to non-diabetic children in doses under 10

units twice a day causes no acceleration of the rate of gain in weight. Doses of 10 units twice a day have a slight accelerating effect and of 15 units twice a day have a somewhat more accelerating effect. The total gains are small. The gains in weight which are induced by these doses disappear within one month after cessation of the insulin. The results do not warrant the injection of large amounts of insulin to non-diabetic children with the dangers of this procedure for the purposes of inducing weight. Results in a control group coincided with those obtained in the insulin-treated group.—M. B. G.

Hyperinsulinism. Wilder, R. M., Internat. Clin. **2:** 1. 1933.

This is a good review of the subject. The onset of hyperinsulinism is insidious. An initial attack of hyperglycemic crisis without loss of consciousness may occur without previous warning, but mild episodes usually precede development of more serious trouble. The course as a rule is usually chronic. The attacks of symptoms appear at irregular intervals. Weeks may pass with little or no inconvenience in the milder cases, whereas in extreme cases hourly feeding, day and night, may be necessary to prevent them. The attacks in the spontaneous disease differ in no important particular from the reactions that are provoked by excessive doses of commercial insulin. The importance of this article is such that the student of the subject had best study this paper in its entirety.—I. B.

Anti-insulin action of the pancreatic juice (Untersuchungen uber eine im Pankreas vorkommende Substanz mit antiinsulinartiger Wirkung). Wohlenberg, W. and K. Müller, Arch. f. exper. Path. u. Pharmakol. **171:** 340. 1933.

The pancreatic juice of eight non-diabetics was obtained by means of a duodenal tube. The diastatic ferments were removed from the pancreatic juice by ultrafiltration. Small amounts of this juice from non-diabetics, usually 2.5-5 cc., injected into fasted rabbits caused increases of blood sugar varying from 17.7 to 55%, whereas the pancreatic juice from diabetics prepared in the same manner caused increases varying from 32.7 to 122.7%.—E. L.

A study of the physiology of the pineal gland (Untersuchungen über die Wirkung der Zirbeldrüse). Engel, P., Ztschr. f. d. ges. exper. med. **93:** 69. 1934.

A study was made of the effect of implants and extracts of pineal gland upon mice, rats and guinea pigs. Extracts were prepared with $Na_2 CO_3$ solution (1% and 0.5%) from pineal glands taken at post-mortem from patients dying of carcinoma, nephritis and infections and were given to a series of 48 castrated female mice. Both the implants and extracts of pineal gland were found to contain an oestrogenic substance as indicated by a positive Allen-Doisy test. A human pineal gland contains about 8 mouse units of this substance. Implants of human pineal gland were given to two castrated immature female guinea pigs. The vaginal smears showed squamous cells on the 6th day. The animals suffered weakness, loss of appetite and paralysis of the hind legs after one week. At autopsy on the 7th day no changes in the uterus were noted. No changes were seen in the seminal vesicles of castrated male mice after 4 and 8 days of implants and injections of extract of pineal gland. Young rats injected with extract of pineal gland from the second day of life showed considerable growth inhibition and in most cases the animals died before the 15th day. General development such as hair growth and the opening of the eyes proceeded at the same rate as in the controls. The possibility of the extract being sufficiently toxic to inhibit growth is not suggested. Injection of extract of pineal gland diminished the chance of take and inhibited the growth of carcinoma implants in mice, although the period of survival of the animals was not diminished. Less influence upon the growth of sarcoma implants by injections of pineal extract was noted.—Evelyn M. Anderson.

Diabetes insipidus associated with pinealoma transplant in the tuber cinereum. Stringer, S. V., Yale J. Biol. & Med. **6:** 375. 1934.

The history and necropsy findings are presented of a white male, 27 years of age who suffered from diabetes insipidus for a period of five months preceding death which followed after a period of hyperpyrexia of 107° F. lasting one week. Generalized signs of an intracranial tumor were absent. The ad-

ministration of pituitrin was at first successful in relieving the diabetes insipidus but gradually this therapeutic measure failed to be effective. At necropsy a pinealoma was found in the pineal body and a transplant of this tumor was present in the region of the tuber cinereum. This metastatic implant destroyed the tuberal group of hypothalamic nuclei and invaded the stalk of the pituitary gland, producing closure of the infundibular canal.
—Author's Abst.

Treatment of functional uterine bleeding with extract of placenta. Gabrielianz, A., Am. J. Obst. & Gynec. **28:** 262. 1934.

An extract similar to that of Collip's was used. Twenty-four patients, ranging from 18 to 48 years of age, were treated. The duration of hemorrhage had been from one month to four years in various cases. Included in the series were cases of menorrhagia, metrorrhagia and polymenorrhea, the last mentioned of which responded most promptly. In one instance of metrorrhagia five months of treatment were required to restore menstruation to normal. In one case overluteinization resulted from the treatment followed by amenorrhea of two months. One patient only failed to respond to treatment. Except for one instance in which the patient became suddenly pale and had palpitation of the heart and tremors of the hand no untoward effects were seen. It is concluded that placental extract is the strongest known luteinizing preparation.
—R. G. H.

The thymus gland and thymic symptoms; an investigation of 1074 new-born babies. Capper, A. and R. A. Schless, J. Pediat. **4:** 573. 1934.

A roentgenological classification of enlargement of the thymus is offered in which type plus 1 represents the slightest breadth enlargement and plus 4 the most marked in width and depth. An enlargement was found in 322 or 30%. Almost 50% or 150 of these belonged to type 1, 76 were in class 2, 51 in class 3 and 45 in class 4. Of the entire group of 1074 babies only 15 or 1.4% showed possible thymic symptoms. In 9 of these there was also some additional conditions which might have been responsible for the symptoms, leaving only 6 infants or 0.56% in whom symptoms could with any certainty be ascribed to an enlarged thymus only. The authors suggest that enlargement of the thymus be divided into that with or without symptoms and that the former be diagnosed only when there is an unmistakable x-ray evidence and all other conditions have been eliminated.—M. B. G.

Hemorrhagic disease of the thymus. Gannon, N. D., Pennsylvania M. J. **37:** 379. 1934.

This case is of interest because of its rarity and atypical symptomatology and unusual findings at necropsy. The patient described was an infant aged ten weeks, apparently well, which suddenly went into collapse, becoming limp, pale, pulseless and dyspneic. Examination and various laboratory tests led to the diagnosis of "small intestinal obstruction." A few hours after the onset of collapse the child temporarily rallied following repeated administration of glucose by hypodermoclysis. Several hours thereafter the infant died. Necropsy revealed the presence of pneumonia in association with a pronounced hemorrhage in the thymus gland which weighed 20 grams. The entire organ was covered with blotches of hemorrhage varying in size from pin point to 1 cm. in diameter extending throughout the organ.—I. B.

So-called thymic death; the pathologic process in thirty-four cases. Waldbott, G. L., Am. J. Dis. Child. **47:** 34. 1934.

This is the report of a pathological study of 34 cases of so-called status thymolymphaticus in which no adequate cause of death had been determined. These were selected from a larger group of 102 cases in which the diagnosis of status thymolymphaticus had been made by various pathologists but the remainder had been excluded because of the presence of other conditions which may have been sufficient to have caused death. The most constant and characteristic changes were present in the lungs characterized by capillary congestion, extravasation of blood cells and edematous fluid. These lesions alternated with areas of emphysema and atelectasis. Hypoplasia of the adrenals and hyperplasia of the lymphoid tissues were present. The author feels that there is a close resemblance between this pathological process and that reported

in anaphylactic shock. Other points of similarity are: (1) Enlarged thymus and lymphoid structures in anaphylactic shock. (2) Enlargement of thymus and lymphoid tissue in asthma. (3) Hyperplasia of the thymus in sudden death following administration of certain drugs. (4) Enlarged thymus in allergic conditions, found by several authors. (5) Sudden death in allergic families. (6) Similarity in constitutional make-up of allergic and thymic individuals. The author advances the theory that death in so-called thymic death may be the result of a primary anaphylactic edema of the lungs and ensuing asphyxia. This theory can be upheld if one assumes (1) that anaphylactic shock may occur from incorporation into the body of non-protein substances and (2) that absorption of shock-producing antigen may take place by other means than by injection.—M. B. G.

So-called thymic hyperplasia; a follow-up study of thirty cases. Waldbott, G. L. and G. E. Anthony, Am. J. Dis. Child. **47:** 34. 1934.

In 30 children whose condition had been diagnosed as "enlarged thymus" by roentgenological examination, a follow-up study revealed that 24 were afflicted with some allergic condition as evidenced by history and skin tests. The possible relationship of thymic enlargement to anaphylactic death is discussed. The authors suggest that thorough allergic studies be made in all cases presenting x-ray evidence of an enlarged thymus.—M. B. G.

Reduction of serum lipase produced by thyroxine and inhibition of this action of thyroxine (Uber den Sturz der Serumlipase durch Thyroxin und dessen Hemmung). Bauer, J. and M. H. Hoffman, Klin. Wchnschr. **12:** 1933. 1933. Abst., J. A. M. A. **102:** 499.

Bauer and Hoffman direct attention to the observations of several Hungarian investigators, who noted that a single subcutaneous injection of thyroxine effects in rabbits a considerable reduction in the lipase content. They point out that their own observations of the extremely low lipase values in patients with exophthalmic goiter correspond to this thyroxine effect, and that the thyroxine test is a simple method for the demonstration of substances that protect against thyroid intoxication. After reviewing some of the contradictory reports about these substances, they call attention to the studies of Anselmino and F. Hoffmann, who succeeded in isolating from the blood and tissues of normal animals a lipoid substance that protects against thyroxine. They relate their own investigations on these problems in rabbits. They were able to confirm that the subcutaneous injection of thyroxine (from 0.25 to 0.5 mg.) causes a considerable reduction in the serum lipase content. Small amounts of the lipoid substance dissolved in oil were fed to the animals for seven successive days. Then they were given injections of 0.25 mg. of thyroxine for a number of days, and these injections did not reduce the lipase content. Later, 0.5 mg. of thyroxine was administered at irregular intervals, but even these amounts did not reduce the lipase as late as 38 days after the feeding with the protective substance. In two animals the protective action of ordinary olive oil was tested, and it was observed that olive oil has a certain protective value but that it is far inferior to the lipoid substance. Other tests revealed that thyroxine reduces not only the lipase but also the fat content.

Coronary artery disease in hypothyroidism. Black, H. A. and R. H. Kampmeier, Colorado Med. **31:** 262. 1934.

Hypothyroidism predisposes to arteriosclerosis. Four cases have been reported in which were found evidence of coronary artery disease. The symptoms of coronary sclerosis may appear with thyroid medication as a result of increasing demand made upon the myocardium to supply the necessary blood flow attendant upon rising metabolism. Fatalities from coronary artery disease may result from the misuse of thyroid extract. Patients being treated with thyroid extract should be carefully observed for evidence of coronary disease, and the use of the medication should be governed according to the individual case.—Authors' Summary.

Surgery of the thyroid in children. Bowen, W. L., Illinois M. J. **64:** 277. 1933.

The earlier the diagnosis of hypothyroidism is made in children the better the results of treatment. In delayed treatment of hypothyroid cases more can

be expected in the improvement of the physical than the mental state. Exophthalmic goiter in children is a more treacherous disease than in adults for the following reasons: It requires a longer period of preparation. The postoperative reaction is apt to be worse. Cooperation cannot be expected. Adolescent goiter is not a normal physical enlargement but may be considered an indication of future thyroid trouble and should be properly treated and watched. —I. B.

Hypothyroidism and cretinism in children; capillary permeability. Bronstein, I. P. and M. E. Milliken, J. Pediat. **4:** 648. 1934.

Capillary permeability was studied by means of the cantharides plaster in 11 children with hypothyroidism and in 45 without any signs of thyroid deficiency. The blister time is the period between the placement of the plaster and the first evidence of its appearance. The permeability ratio is the percentage of blister protein divided by the percentage of serum protein. This coefficient indicates the actual degree of response of the capillaries endothelium to a direct stimulus. The inflammatory index is the permeability ratio divided by the blister time. This coefficient reveals the effect of the autonomic tonus. In the non-thyroid group the average time for blister formation was determined at 5.4 hours. The average permeability was 66.6. The average inflammatory index was 13.85%. In this group of non-thyroid children blister determination indicates that decreased permeability may be associated with increased blister time. No reciprocal relationship exists between the degree of permeability and the length of blister time. The average time for blister formation in thyroid defective children while under treatment with thyroid extract was that usually within the average time of the non-thyroid group. Withdrawal of the extract results either in an increase in the time or an inability to form a blister. The variability of the permeability and the inflammatory indices in children in the thyroid group and the relation between these and blister time is similar to that found in the non-thyroid group.—M. B. G.

Contribution to the problem of Basedow's adynamia (Beitrag zum Problem der Basedow'schen Adynamie). Charvát, J. and K. Silink, Wien. med. Wchnschr. **84:** 568. 1934.

Adynamia is almost as often present in exophthalmic goiter as is the increased B.M.R. Its intensity, however, does not correspond with that of the other clinical symptoms. The weariness of the muscles may last, even after the general improvement, or it may disappear before the manifestation of the general improvement. The beginning of the weariness of the muscles may appear independently, and in a series of cases the authors were able to observe adynamia as a first sign of hyperthyroidism. They were unable to find a regular correlation between the B.M.R. and the efficiency of the muscle function and are of the opinion that the adynamia in Basedow's disease cannot be explained simply as a consequence of increased oxidation in the muscles. —G. L. Banay.

Relationship between blood cholesterol and increased metabolism from dinitrophenol and thyroid. Cutting, W. C., D. A. Rytand and M. L. Tainter, J. Clin. Investigation, **13:** 547. 1934.

From previously reported data, a significant correlation between basal metabolic rate and blood cholesterol concentration has been established for human subjects. This correlation was not present in 28 subjects when the metabolism was raised an average of 28% by dinitrophenol in dosages of from 100 to 400 mg. Therefore, the changes in blood cholesterol present in thyroid disease are not related directly to the metabolic rate, but to other actions of thyroid secretion. The stimulation of metabolism by dinitrophenol was as great in patients with initial metabolic rates below minus 15% as it was in those with higher rates, which indicates that the drug may increase depressed as well as normal metabolism.—Authors' Abst.

Experimental study of action of 3-5 diiodotyrosine in treatment of hyperthyroidism (Étude expérimentale de l'action de la 3, 5 diiodo-tryrosine dans le traitement de l'hyperthyroïdie). Delcourt-Bernard, E., Rev. belge sc. méd. **6:** 1. 1934. Abst., J. A. M. A. **102:** 1724.

Delcourt-Bernard found that from the physiopathologic point of view diiodotyrosine exerts a beneficial effect on the increased respiratory exchange

in the course of hyperthyroidism, whatever the clinical form of the disease may be. Of 12 cases observed by the author, eight presented a lowering and four an elevation of metabolism. The mechanism of the action of compound solution of iodine differs from that of 3-5 diiodotyrosine in that, when the former lowers the metabolism, it seems to act more on aeration than on the consumption of oxygen. When it determines an elevation of the rate of exchange it acts more on the consumption of oxygen than on aeration. Diiodotyrosine, on the other hand, has more of a tendency to lower the consumption of oxygen when it lowers the exchange and to act equally on the two factors when it raises the metabolism. The most important and most frequent increases in the weight of the subjects treated are to the credit of compound solution of iodine. No differences were observed in the action of these two substances on the pulse or on other symptoms of hyperthyroidism. Except for the equivalence of doses, the author does not grant to diiodotyrosine the peculiar value that certain German authors have attributed to it and cannot compare it, as many Americans do, to a solution of inorganic iodine acting only on account of the iodine that it contains. From the therapeutic point of view, diiodotyrosine is easily absorbed and well tolerated in the prescribed doses of 0.1, 0.2 and 0.3 gm. daily. Without possessing the efficacy or the ease of administration of compound solution of iodine, diiodotyrosine may nevertheless be of service in replacing compound solution of iodine or in obtaining a gastric tolerance momentarily diminished with regard to the latter or in being associated with compound solution of iodine after having acted previously by itself. The fact that in certain cases it may determine an elevation of the respiratory exchange with acceleration of the pulse demands a close supervision of cases treated with it.

The influence of the season of the year and the parenteral application of thyroid gland on the metabolism of the mollusc Linnaea palustris. Dusková, V., Spisy lák. fak Masaryk. Univ. **11**: 1. 1931.

Thyroid gland substance or thyroxin administered to the mollusc Linnaea palustris caused an increased metabolism. The normal metabolism of the mollusc is highest in June to August and lowest, one-tenth that of summer, in November to January.—Author's Abst.

The mitotic index of the thyroid gland in the guinea pig and rat. Friedman, Hilda and L. Loeb, Anat. Rec. **59**: 5. 1934.

The average number of mitoses and the number of cells in the thyroid glands of guinea pigs and rats were counted. There is an increase in the number of cells with increasing weight of the animals. If we compare guinea pigs and rats of similar weight, the number of cells in the thyroid glands of these two species is similar. The mitotic index of the thyroid gland of the guinea pig is much higher than that of the rat, notwithstanding the fact that the anterior pituitary gland of the rat is more efficient in causing stimulation of the thyroid gland of either guinea pig or rat than that of the guinea pig. This suggests the possibility that in addition to differences in the mutually balancing effects of the hormones of the anterior pituitary and thyroid glands factors inherent in the constitution of the thyroid glands in different species determine the activity of these organs and their responsiveness to anterior pituitary hormone. While the mitotic index of the epidermis is of a similar order in guinea pig and rat, the relation of the mitotic index of the thyroid gland to that of the epidermis is much lower in the rat than in the guinea pig.—Authors' Abst.

Diagnostic importance of blood volume and cardiac output studies in a borderline case of thyrotoxicosis. Goldbloom, A. A., M. Clin. North America, **17**: 279. 1933.

This paper points out that in hyperthyroidism a high pulse pressure and an unduly rapid circulatory time are pathognomonic. Also in this condition the arteriovenous oxygen difference is practically on a constant level. In the differential diagnosis it is well to remember that variable basal metabolic rates, extending as high even as above plus 60% are not necessarily of thyrotoxic origin but may be due to imbalance of the vegetative nervous system.—I. B.

The treatment of hyperthyroidism. Goodwin, G. M., M. Clin. North America, **17:** 1171. 1934.

This is an evaluation of the medical, surgical and x-ray treatment of hyperthyroidism. The author believes surgery to be the treatment of choice. He believes x-ray therapy of value in mild cases kept under frequent observation, particularly when mild toxemia continues after thyroidectomy.—I. B.

Effect of compound solution of iodine on hyperthyroidism and dysthyroidism (Über die Wirkung der Lugolschen Lösung auf die hyper- und dysthyreotische Schilddrüse). Irsigler, F. G., Deutsche Ztschr. f. Chir. **242:** 689. 1934.

The clinical effect and the histological changes in the thyroid, following administration of Lugol's solution, were studied. The material on which this paper is based consisted of 14 goiters which were removed from patients with thyrotoxicosis, at Erlangen, Bavaria. Three different groups of goiters were encountered; (1) diffuse colloid goiters with involution, (2) goiters with large areas of hyperplasia, (3) microfollicular colloid goiters and (4) typical exophthalmic goiters. The author concludes that iodine produces first a stimulating, later a paralyzing effect on the acinar epithelium of the hyperplastic thyroid. Regeneration may take place. The histologic changes in exophthalmic goiter, following administration of Lugol's solution, are characteristic. There is, however, no definite relationship between anatomical changes and clinical improvement.—C. A. Hellwig.

Functional disturbance of thyroid gland in chronic industrial mercurialism. Kazakevich, M. A., Klin. med. **11:** 1247. 1933. Abst., J. A. M. A. **102:** 1266.

Kazakevich investigated 133 workers in a mercury plant. Of these 48 were men and 85 women; 60.9% of the group were between 20 and 30 years of age. The duration of occupation was from six months to three years. Manifestations of a thyrogenous character with an enlargement of the thyroid were observed in 57.3%. The incidence of vasomotor neurotic states of thyrogenous character raises the percentage to 88.9. The following constituted characteristic complaints: irritability, somnolence, poor memory, disturbed sleep, headache, tremor of the extremities, particularly of the fingers, flushes, sweating, palpitations, falling out of hair, increased salivation, bleeding gums, weakness and apathy. Among the characteristic objective signs were tremor of the eyelids, dilated pupils, exophthalmos, absent or weakened pharyngeal and conjunctival reflexes, tremor of the fingers, red dermographism, increased sweating and salivation, enlarged and tender thyroid, and falling out of hair. Double color line of the gums, pale skin and mucous membranes, arterial hypotonus and labile pulse were rather frequent signs in chronic mercurial poisoning. Enlargement of the cervical and inguinal lymph nodes was present in almost all cases. In the beginning the nervous system reacts to mercurial intoxication with a vasomotor neurosis, which gradually develops into hyperthyroidism if the intoxication continues. The highest values of mercury excreted in the urine were found in adolescents up to the age of 20, and disturbances of the thyroid function and of the vegetative nervous system occurred in them with greatest frequency. The incidence of hyperthyroidism was higher in women than in men. The author urges a number of preventive measures for the removal of mercurial vapors, exclusion of workers below the age of 20, and the consideration of limiting in some cases the period of employment to six months at one time. Administration of salts of iodine and of bromine because of the readiness with which these halogens combine with mercury is recommended just as soon as the first signs of mercurial irritation appear.

Fatigue in school children. Kerley, C. G., J. Pediat. **4:** 322. 1934.

This study covers a group of 32 school children who were treated for fatigue, an inability to carry on the usual school work and a lack of general interest. In one group there was a favorable response to adequate rest habits, the correction of minor gastro-intestinal disorders and minor disabilities. The second group consisted of 10 children, 7 boys and 3 girls. All showed a low B.M.R. without any other suggestions of thyroid deficiency. Improvement followed the administration of small doses of thyroid extract.—M. B. G.

Compensatory hypertrophy of thyroid in hypophysectomized toads (Hipertrofia compensadora de la tiroides en los sapos hipofisoprivos). Magdalena, A., Rev. Soc. argent. de biol. **10:** 411. 1934.

In Bufo arenarum (15 males and 15 females) three-fourths of the thyroid gland was extirpated and 2 days later the anterior pituitary lobe removed in 18. The remaining thyroid tissue was examined microscopically 30 days later. Those with intact anterior pituitary had a typical hypertrophy of the thyroid; a great number of small vesicles with high epithelium and reabsorption of colloid, which was fluid and vacuolized. Those in which the anterior pituitary had been removed had quiescent thyroid tissue: low epithelium, large vesicles, abundant colloid with few or no vacuolae.—J. T. L.

Metabolism in hyperthyroidism and hypothyroidism. Palmer, W. W., M. Clin. North America, **17:** 1159. 1934.

The basal metabolic test for thyroid activity may involve considerable error. Occasionally a normal person presents a B.M.R. well outside the range set as normal for the test. In undernourishment the metabolism is lower while in the well-nourished it may be higher than the conventional standard. Also, the mental status, the muscular tone and other factors may account for slight or moderate deviations in metabolic readings. A slightly elevated B.M.R. may be found in hypertension, malignancy, pregnancy, acromegaly, the leukemias, polycythemia, and infections. A lowered B.M.R. is found in the anemias, Addison's disease, some skin diseases, pituitary tumor and diabetes. A patient typically myxedematous may show repeated metabolic readings within normal limits. Thyroid substance may be employed as a therapeutic test under B.M.R. control to aid in diagnosis in the presence of low readings without clinical features of hypothyroidism. The normal variation of the B.M.R. in adolescence is greater than among adults, usually on the low side. The author condemns the indiscriminate administratiion of thyroid. In hyperthyroidism, the B.M.R. is of relatively little service in diagnosis as compared with its use in determining results of treatment. The test is very useful in detecting cases of heart disease masquerading as hyperthyroidism.—I. B.

Cholesterol partitions of the blood in myxedema (cretinism). Schwarz, H. and A. Topper, J. Pediat. **3:** 242. 1933.

This paper reports a study of the proportion of free cholesterol to ester in 11 cases of myxedema. The ester was reduced in all but 4 cases so that the normal ratio of combined ester to free cholesterol was reversed, a reversal which the authors claim occurs only in hypothyroidism and in hepatic dysfunction. It seems that this ratio may become normal after intensive treatment with thyroid extract. It is possible that in the determination of the ester to free cholesterol ratio we may have a diagnostic aid to thyroid therapy in myxedema and hypothyroidism.—M. B. G.

Congenital goiter; thyroidectomy at 18 months. Williamson, G. R., J. Pediat. **2:** 458. 1933.

The outstanding features of this case report are: Cyanosis and enlargement of the neck at birth, a latent period of thirteen months, rapid increase in size from then on until the time of operation with an increase in severity of symptoms; thyroidectomy at 18 months, uneventful recovery and no history of goiter in the family. The gland on section showed a diffuse adenomatous hyperplasia.—M. B. G.